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STUDIES ON THE *BRACON HEBETOR–BREVICORNIS* COMPLEX (HYMENOPTERA: BRACONIDÆ)

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Introduction

The rôle played by *Bracon brevicornis* Wesmael and *B. hebetor* (Say) as ectophagous larval parasites of many well-known pests is an important one. The number of hosts on which these ectoparasites have been recorded is very large indeed, as evidenced by the available literature, both in India as well as abroad. Notwithstanding the very extensive work by a multitude of entomologists done on the biology of these forms, it is not known for certain whether *B. brevicornis* and *B. hebetor* should be regarded as different species or that one is merely the variety or race or sub-species of the other. The latter is the conclusion. Puttarudriah and Channa Basavanna (1956) arrived at, on the basis of their studies of the morphological characters (genitalia

included) and the experiments stated by them to have been made to interbreed the forms. However these workers, *viz.*, Puttarudriah and Channa Basavanna did not give a detailed description of the male genitalia of the two forms. Indeed the only reference thay made to the external male genitalia, of vital importance in specific determination not only in the Hymenoptera but also in most other orders of Insecta, is in the following words: " An examination of the male and female genitalia of a number of specimens of *hebetor* and *brevicornis* (inclusive of those reared on *Pyrausta* and *Nephantis*) *revealed almost no distinct difference between the two species* " (t.c.: p. 189) (Italics of the present authors).

The work carried out by the present authors seems to point to an entirely different conclusion, *viz.*, that *B. brevicornis* and *B. hebetor* are two separate or discrete species. The studies made were of two kinds: (1) morphological and (2) biological, the latter comprising the experiments conducted to find out whether the two forms interbred.

MATERIAL AND METHODS

Individuals of *Bracon brevicornis* were obtained from the culture of this parasite being maintained in the Parasite Laboratory of the Entomology Division, Indian Agricultural Research Institute, New Delhi. Early last year (1956) a few live pupæ of *B. hebetor* were received from the Division of Entomology, Mysore Agriculture Department, Bangalore, and a regular culture of this parasite is also being maintained in this laboratory since that time.

For studying the male genitalia, the cut abdominal tips were kept overnight in 10% KOH solution, washed thoroughly in water, dissected under binoculars and then mounted in Canada balsam after being passed through acetic acid and carbol-xylol mixture (3 parts xylol: 1 part carbolic acid). No staining with carbol-aniline, etc., was found necessary as the various components were sufficiently well scleritised.

As regards the hybridisation experiments, pupæ of each form were kept in separate tubes (one pupa only in one tube) and, when adults emerged, reciprocal pairs were released in small glass jars covered with muslin cloth and were provided with moistened split raisins. Two full-grown *Corcyra* larvæ were exposed to the female of each pair over a period of 24 hours. The parasitised *Corcyra* larvæ were transferred to paired petri-dishes after this period of time and fresh larvæ were exposed. This was continued till the female of each pair died.

To serve as a sort of control or check, males and females of the same species (1♂ *hebetor* × 1♀ *hebetor*, 1♂ *brevicornis* × 1♀ *brevicornis*) were also paired and provided with *Corcyra* larvæ. The progeny developing on the *Corcyra* larvæ in both sets of experiments were studied as to the sex of the individuals. All experiments were conducted at 25° C. and 70% R.H.

Morphological Studies

(*A*) *Colour characters*

A study of the colour characters of about two hundred individuals each of the two forms showed that no great reliance could be placed on these characters. Richard and Thomson (1932), Cherian and Margabandhu (1949) arrived at the same conclusion as this during the course of their studies. This is not surprising since the inadequacy of chromatic difference and variations has been demonstrated in many other groups of the Insecta. Moreover, as Narayanan *et al.* (1954) showed, even ontogenetic colour changes can be induced in the case of *B. brevicornis* by subjecting the pupæ to different temperatures.

(*B*) *Number of antennal segments*

(1) *B. hebetor*.—(*a*) Males—From Table 1 A (*i*) it will be seen that the highest number of antennal segments in a single individual was 25,

Table 1 A

B. hebetor

(i) Males		(ii) Females	
Antennal segments	Number of specimens	Antennal segments	Number of specimens
25	4	18	5
24	39	17	45
23	25	16	0
22	8	15	0
21	4	..	..
20	3	..	..
Total number in the lot examined ..	83	Total number in the lot examined	50

among the specimens examined by the authors, while the lowest was 20. Table 1 C: A shows the number of males with an unequal number of antenna segments.

(*b*) Females—The highest number of antennal segments for a single individual was 18, while the lowest number was 17. Out of 50 specimens examined only 5 had 18 antennal segments: the others had only 17 [Table I A (ii)]. Of the 50 females examined, none showed a difference in the number of segments between the two antennæ (Table I C: B).

TABLE I B

B. brevicornis

(i) Males		(ii) Females	
Antennal segments	Number of specimens	Antennal segments	Number of specimens
25	0	18	0
24	3	17	8
23	25	16	27
22	30	15	9
21	4	..	..
20	2	..	..
Total number in the lot examined ..	164	Total number in the lot examined ..	44

(2) *B. brevicornis.*—(*a*) Males—From Table I B (i) it will be seen that the highest number of antennal segments in a single individual was 24, and the lowest number was 20. The majority of specimens had 22 segmented antennæ.

(*b*) Females—It is seen from Table I B (ii) that none of the females had more than 17 segments in the antenna, while the lowest number of antennal segments in an individual was 15. The majority of individuals had 16 antennal segments.

(*C*) *Male genitalia*

(*a*) *B. hebetor.*—The entire male genitalia of this species are illustrated on Plate II, Fig. 1, while the phallus (as separately dissected and mounted) is shown in Plate II Fig. 2.

TABLE I C

B. hebetor: A. Males

Total number of specimens examined	Number of individuals with unequal number of segments in the two antennæ			
83	Number of segments in the right antenna	Number of segments in the left antenna	Number of segments in the right antenna	Number of segments in the left antenna
	24	23	22	21
	23	24	21	20
	23	24	24	23
	22	23	25	24
	23	24	21	22
	22	23	22	23
	23	24	24	25
	24	23	22	20
	22	23	22	23
	23	22	24	23

B. Females

Total number of specimens examined	Number of individuals with unequal number of segments in the two antennæ
50	Nil

(*b*) *B. brevicornis.*—The male genitalia are illustrated on Plate II, Fig. 3 and the phallus is separately illustrated in Plate II (Fig. 4).

Since the authors propose to describe the male genitalia of not only these two forms but also of other available species of the genus *Bracon* Fabricius, both Indian and exotic, elsewhere in detail, exhaustive descriptions of the male genitalia of the two forms are not given here. However, the illustrations clearly show the salient and quite marked differences between the male copulatory organs of the two forms not only in the gross parts but also in a minute component, like the phallus.

(*D*) *Wings*

The fore and hind wings of *B. hebetor* are illustrated on Plate I, Figs. 1–2 and those of *B. brevicornis* in Figs. 3–4.

BIOLOGICAL STUDIES

The details of the experiments to find out whether the two forms mated to produce a " bi-sexual " progeny or not have been described earlier in this paper. In addition to these experiments, males and females of the same

TABLE I D

Bracon brevicornis: A. Males

Total number of specimens examined	Number of individuals with unequal number of segments in the two antennæ			
	Number of segments in the right antenna	Number of segments in the left antenna	Number of segments in the right antenna	Number of segments in the left antenna
164	20	21	22	23
	21	22	23	22
	21	22	21	22
	23	22	23	22
	23	22	20	19
	22	23	22	23
	23	22	..	..

B. Females

Total number	Number of individuals with unequal number of segments in the two antennæ	
	Number of segments in the right antenna	Number of segments in the left antenna
44	16	17
	15	16
	17	16
	17	16
	17	16
	15	16

form were also caged together and their progeny examined. These latter experiments, as explained earlier, were designed to serve as 'controls' for the experiments to find out whether the two forms interbred successfully.

The results of the 'reciprocal-cross' experiments are contained in Table II: 1, while those of the 'controls' are shown in Table II: 2. It will be seen from Table II: 1 that the progeny of all the reciprocal crosses was purely males, whereas females were present in some numbers in the progeny of the crosses of the same species (Table II: 2).

Discussion of the Results Obtained

Puttarudriah and Channa Basavanna (1956) made the following statement, while reviewing the extant literature on the host range of the two forms: "Richards and Thomson (1932) reviewed the host records of the two species and it is interesting to note that, while giving the synonymy of *B. hebetor*, *B. brevicornis* was also included though they considered the latter separately and regarded it as established that *B. hebetor* is mainly a domestic species and *B. brevicornis* lives out of doors with of course a few exceptions in both cases" (t.c.: p. 183).

Puttarudriah and Channa Basavanna seem to imply that by including the *brevicornis* of certain authors in the synonymy of *hebetor* Say, Richards and Thomson synonymised *hebetor* and *brevicornis* out right. A more careful study of that part of the work by Richards and Thomson (1932) (p. 225) shows that what the authors meant was merely this: that certain authors [like Kirby (1884), Schmiedeknecht (1896), etc.] record as *brevicornis* certain forms which should be more correctly assigned to the species *hebetor*. Hence there was nothing unusual or 'interesting' about this part of the paper by Richards and Thomson (1932).

The number of antennal segments has been regarded as one of the chief criteria on which the two forms can be separated—for example, in the keys given by Muesebeck (1925) who stated that in *hebetor* the antenna of the female had 13 to 15 segments and that of the male had 18 to 23 segments, while in *brevicornis* the corresponding numbers were 17 to 19 ("very rarely 16-segmented") and 20 to 27. Lal (1947) stated in this connection: "The table of characters (as given in Muesebeck's key) for *hebetor* should stand, but for *brevicornis* the antennal segments in the female may range from 16 to 21 (t.c.: p. 88). However, there is reason to believe that there is considerable variation in the number of antennal segments, both in the male and female in the family Braconidæ, particularly in species and genera with a comparatively large number of antennal segments as is the case with the genus *Bracon* Fabricius.

TABLE II

Cross		Number of progeny	Sexes		Cross		Number of progeny	Sexes	
			Males	Females				Males	Females
Hebetor ♂	1.	30	30	0	*Hebetor* ♂	1.	15	8	7
Brevicornis ♀	2.	25	25	0	*Hebetor* ♀	2.	13	9	4
	3.	20	20	0		3.	12	9	3
	4.	26	26	0		4.	20	12	8
	5.	16	16	0		5.	22	15	7
	6.	13	13	0		6.	19	14	5
Hebetor ♀	1.	24	24	0	*Brevicornis* ♂	1.	20	10	10
Brevicornis ♂	2.	22	22	0	*Brevicornis* ♀	2.	22	14	8
	3.	14	14	0		3.	11	7	4
	4.	16	16	0		4.	17	12	5
	5.	21	21	0		5.	18	14	4
	6.	28	28	0		6.	19	10	9

The results of the observations made on this point by the present authors are, however, very dissimilar to those of both Muesebeck (1925) and Lal (1947). We found that in *hebetor* the majority of the females had 17-segmented antennæ and only 5 out of 50 females had 18-segmented antennæ, whereas in the males the highest number of antennal segments was 25 (4 males out of 83 examined) and the lowest number was 20, the majority being with 24-segmented antennæ. As regards *brevicornis* the present authors observed that the highest number of antennal segments in the female was 17 (8 out of 44 females examined), the lowest number 15 (9 out of 44 females examined), the majority of the females being with 16-segmented antennæ, whereas in the males of the same species (*brevicornis*) the highest number of antennal segments was 24 (3 out of 164 males examined), the lowest number 20 (2 out of 163 males examined), the majority of the females being with 22-segmented antennæ.

Our observations confirm those made earlier by Puttarudriah and Channa Basavanna (1952), regarding the inequality of the number of the segments in the two antennæ of the same individual of a species. Two lots of specimens of the two species were examined and Tables I C & I D give an idea of the results obtained. It will be seen that quite a fair percentage of the specimens examined displayed this inequality. From Tables I C: A & B and I D: A & B an idea of the relative preponderance of this inequality of segmented is seen with respect to the two sexes.

Some other interesting observations were made by the present authors regarding antennal characters in the two species. They are briefly: (1) That the number of antennal segments is not directly correlated with the size of the individual. Thus some specimens has as many as or even more antennal segments than specimens appreciably bigger than them in size. (2) The inequality in the number of antennal segments in the two antennæ of the same individual was often traceable to a partial fusion of two neighbouring segments or rather their imperfect delimitation during development.

However, there were specimens in which this segmental inequality was due to a segment being clearly less in one antenna as compared to the other and not due to incomplete or partial fusion of two segments. Another interesting observation we made was that while in *hebetor* the inequality between the number of segments of the two antennæ of the same individual was very rare in the males but quite frequent in the females. While in case of *brevicornis* it was as frequently encountered in the males as in the females. (Tables I C: A, B & I D: A, B).

In view of the foregoing, Muesebeck's key used, with slight modifications, so far to distinguish *hebetor* and *brevicornis* does not seem to apply fully at least to the large number of specimens examined during the course of the present investigations. We are not sure as to how to explain the difference in the results obtained in this regard by Muesebeck and those obtained by us. The same applies with equal force to the results obtained by Puttarudriah and Channa Basavanna (1956).

As regards the external male genitalia (Plate II, Figs. 1–4), the two species differ in the shape of the basal ring, the volsellar plates and the phallus—which, in each case, was carefully separated and mounted individually.

The wings of the two species (Plate I, Figs. 1–4) show considerable individual variation, especially in regard to the extent to which M_1, m—M_2 and M are marked. An interesting observation made was the " sexual dimorphism " obtaining in both the forms in regard to the degree of differentiation of the vein M. In the males of both the forms, this vein (M) was appreciably less marked than in the case of the females. The venation of the two forms is very similar, but the wings of *B. hebetor* are somewhat narrower and more elongated than those of *B. brevicornis*.

Our experiments to find if *hebetor* and *brevicornis* can interbreed, yielded only negative results, inasmuch as the progeny of each of the 12 reciprocal crosses was all male. In contrast to this, males and females of either species when paired produced both males and females in their progeny, under the same conditions of temperature and humidity (25° C. and 75% R.H.). It may be stated in this connection that there were repeated attempts at copulation in the individuals of the reciprocal crosses, though such attempts were obviously futile, inasmuch as the resulting progeny was only males.

The present problem is admittedly complexer than what it seems to be. We had sent the manuscript of this paper to Dr. C. F. W. Muesebeck, Washington Museum, who is the world's foremost authority on the family Braconidæ. He made some comments thereon which we are reproducing below with his kind permission.

" The problem is a most interesting one, and I thank you for the opportunity of examining your manuscript, although I am very much perplexed by the data you have presented. It is difficult for me to believe that you had the true *hebetor* in your experiments. Say's type had 14- segmented antennæ, and in long series available to me here (including material from various parts of the world, and from such hosts as *Ephestia*, *Plodia* and *Sitotroga*) the vast majority of females have 14 segments; many more have 13 segments in the antennæ than 15, and only three specimens of the very

large number examined have 16. From the data you have presented I would be inclined to consider all your material to be *brevicornis* or segregates of that species. Dr. R. I. Sailer, of our staff, who has been conducting genetic studies in Hemiptera for several years, and to whom I showed your manuscript, suggested that there may be several sibling species in this *hebetor-brevicornis* complex with varying degrees of reproductive isolation.

Wesmael's type of *brevicornis* has 17-segmented antennæ, and the males in his type series have the antennæ 20 to 26-segmented. Our material of *brevicornis* from Europe agrees beautifully with the type series. In the female sex the number of antennal segments ranges from 16 to 18, most of the specimens having 17 or 18; in the males the antennæ are 20 to 26-segmented as in the types. In view of this I am somewhat confused by the numerous specimens I have examined that were reared in India from the pink bollworm and from *Chilo zonellus*. These series seem to be intermediate between *hebetor* and *brevicornis*. In more than 70% of the females the antennæ are 16-segmented; in less than 5% are they either 14-segmented or 17-segmented. Though obviously intermediate the females seem to approach *brevicornis*; but the males are more like *hebetor*, having the antennæ 21 or 22-segmented. It is quite possible, of course, that the apparent differences represent host influences."

From the above-quoted remarks of Dr. Muesebeck's it is clear that there is some ground to doubt the true identity of the *hebetor* specimens involved not only in our own experiments and observations but also in those of Puttarudriah and Channa Basavanna (1956). Say's type of *hebetor* had 14-segmented antenna, and Muesebeck himself states that the majority of the females examined by him had 14-segmented antenna, quite a few had 13, and only a very few had 15 or 16-segments. This is at variance with our observations as also those of Puttarudriah and Channa Basavanna (Table III, p. 188 t.c.).

In view of this, we are constrained to remark that either the original *hebetor* has undergone a large number of mutations and has started showing considerable variation in various morphological characters after having spread to many different climes since it was first described by Thomas Say; or that the species used by Puttarudriah and Channa Basavanna and ourselves is not the true *hebetor*, but is a yet unidentified sibling species from amongst the *hebetor-brevicornis* complex very near the true *hebetor*. It is interesting to note in this connection that this species was identified as *hebetor* by the Commonwealth Institute of Entomology according to Puttarudriah and Channa Basavanna (t.c.: p. 190) and that we got some live pupæ

of this species from Dr. Puttarudriah and it was from this nucleus that a culture was begun in our laboratory.

The passages quoted from Dr. Muesebeck's letter to one of us (E. S. N.) also show the existence of very considerable variation in the number of antennal segments of *brevicornis* as well. How far these differences represent host-influences is beyond the scope of the present work, but we might add here that the *brevicornis* culture being maintained in our laboratory on *Corcyra cephalonica* was started from material identified by Dr. Muesebeck in the year 1954.

In view of the foregoing discussion we have but to admit that the present investigations are only a contribution towards the solution of the admittedly very interesting but puzzling problem of the *hebetor-brevicornis* complex. Light may be thrown on this by a future study of the chromosomes of the species concerned. Such a study will also be, in a way, the final word on the identity of discreteness of *B. hebetor* and *B. brevicornis*. This is not surprising in view of what Muesebeck himself wrote in 1925 regarding the genus *Bracon* (or *Microbracon* Ashmead, as it was then known): " In few groups of the Braconidæ is there found so wide a range of variation within species as in *Microbracon*. Practically all characters, many of them excellent characters in other groups, vary greatly in this genus " (t.c.: p. 5).

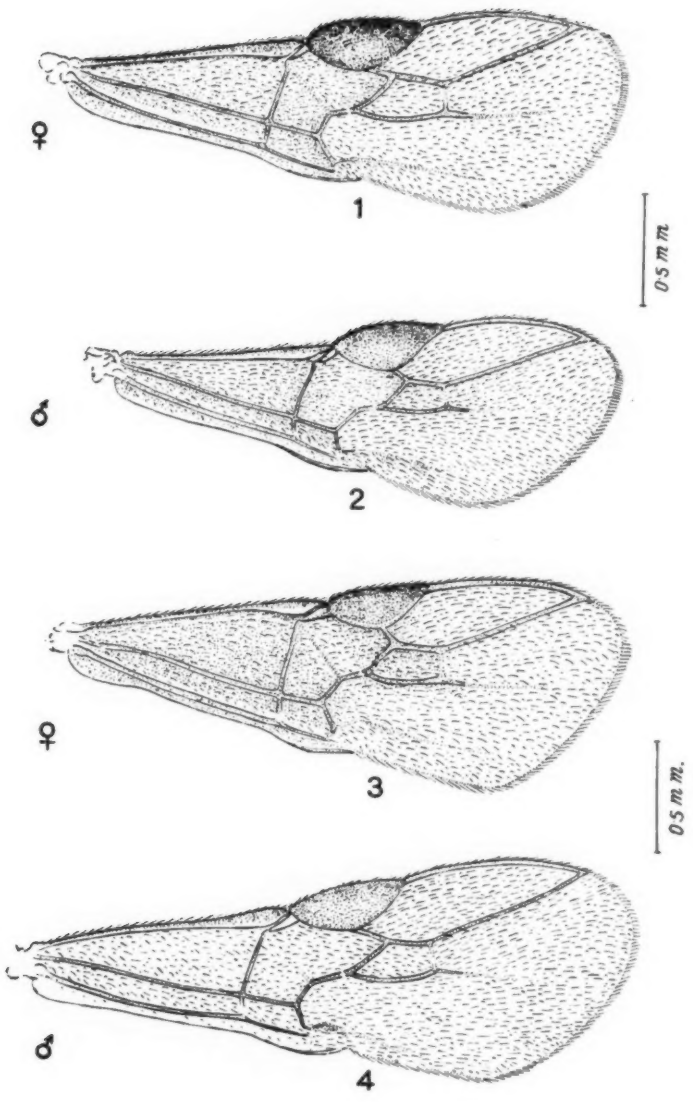
SUMMARY

This work discusses the views of various authors as to whether *Bracon hebetor* (Say) and *Bracon brevicornis* (Wesmael) are two separate species.

The fairly detailed studies made during the course of the present investigations in regard to the antennæ, the external male genitalia and the experiments conducted to find if the two forms successfully mated to give rise to a progeny consisting of both males and females, have been made. The inequality in the number of antennal segments in the individuals has been studied in detail. It is suggested that there is a complex of more than two species and only a thorough cytological study could solve the problem.

ACKNOWLEDGEMENTS

We are indeed grateful to Dr. C. F. W. Muesebeck, U.S. Nationa Museum, Washington, for going through the manuscript and making some very valuable suggestions. The authors also wish to express their thanks to Dr. B. P. Pal, Director, I.A.R.I., for his keen interest and encouragement.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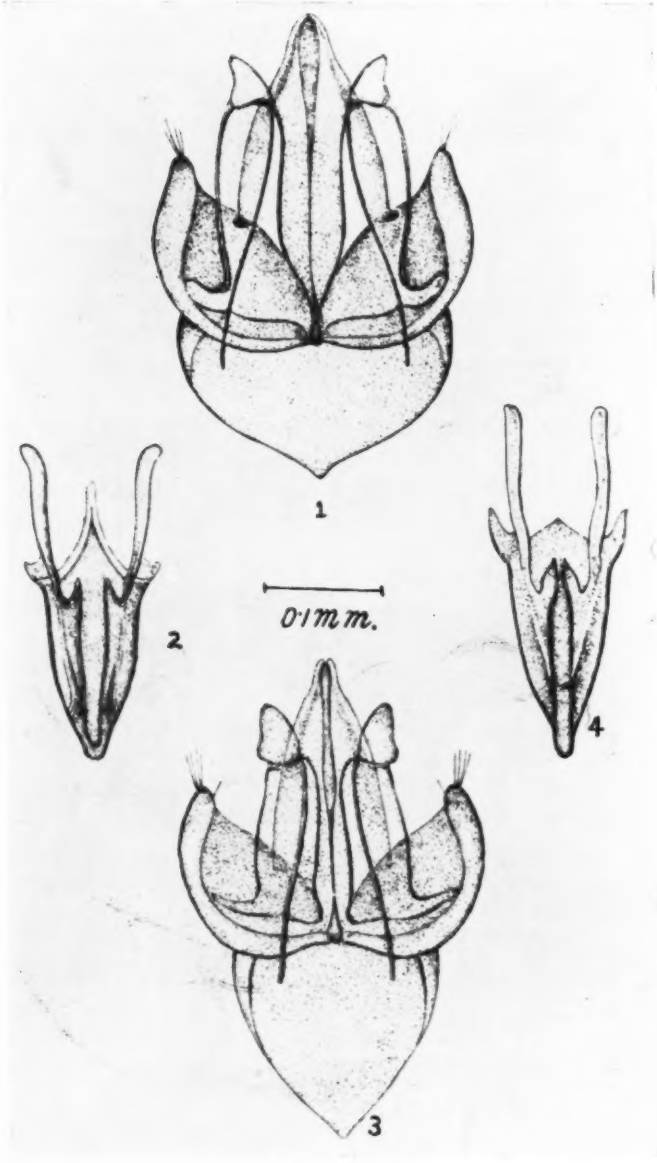
Bracon hebetor



Bracon hebetor

Bracon brevicornis

Bracon brevicornis

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* Not consulted in original.

Explanation of Plates

Plate I

Fig. 1. Fore wing of *Bracon hebetor*, female.

Fig. 2. Fore wing of *Bracon hebetor*, male.

Fig. 3. Fore wing of *Bracon brevicornis*, female.

Fig. 4. Fore wing of *Bracon brevicornis*, male.

Plate II

Fig. 1. Male genitalia, *Bracon hebetor.*

Fig. 2. Phallus, *Bracon hebetor.*

Fig. 3. Male genitalia, *Bracon brevicornis.*

Fig. 4. Phallus, *Bracon brevicornis.*

STUDIES ON THE NUTRITION OF RICE PLANT (*Oryza sativa* L.)

Part II. A Comparative Study of Nitrogen Requirement of *Indica* and *Japonica* Varieties of Rices

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Two geographical races of species *O. sativa* are recognised, the *indicas* cultivated in the tropics and the *japonicas* in the sub-tropics and warm temperate regions beyond 30° north and south latitude. *Indicas* are generally considered low yielders than *japonicas*. Grist (1953), summarising the normal average yield of rice in various countries, has concluded that the average yield of rice in countries where *japonica* varieties are grown is higher than that of countries growing *indica* varieties. Apart from high yield obtained with *japonica* rices, they, unlike the *indicas*, respond to heavy dressing of nitrogeneous fertilizers. Baba (1954) from Japan has reported that *japonica* varieties are suited for heavy manuring conditions because, at high nitrogen supply, they utilize nitrogen more efficiently than *indica* varieties. Ishizuka and Tanaka (1955), from a series of water culture studies, have reported 60 p.p.m. nitrogen as optimum required for yield of *japonicas*. Recently, authors (Tanaka *et al.*, 1958) found that nitrogen requirement for *indica* under growth conditions in India, for optimum yield lies between 20–40 p.p.m. of nitrogen. It is also well known that average nitrogen level used for rice crop in Japan is about 80–90 kg./ha. as compared to 30–40 kg./ha. used in India. An experiment was therefore laid out to study the comparative influence of increasing nitrogen levels on growth, yield and nitrogen assimilation by two varieties under growth conditions obtainable in India, in order to elucidate reason for differential nitrogen response by these varieties.

Material and Methods

Investigations were undertaken during the second crop season (December–April), a time most suited for growth of *japonica* varieties at Cuttack. Two varieties used for comparison were *Ptb* 10 (*indica*) and *Aikoku* (*japonica*). (Hereafter in the text *Ptb* 10 will be referred to as *indica* and *Aikoku* as *japonica*). Seeds were sown in seed pans on December 24 and seedlings were

transplanted after twenty days of growth on January 14, in pots containing eleven litres of culture solution. Three seedlings were planted per pot. Culture solution used for growth contained 20 p.p.m. each of P_2O_5, K_2O, CaO and MgO with traces of iron, manganese and silica. Initial pH of culture solution was adjusted to 4·8 with dilute sulphuric acid (Tanaka *et al.*, *l.c.*). Nitrogen was given in the form of ammonium nitrate in graded doses of 0, 5, 20, 60 and 150 p.p.m. Tank water containing a total of about 3 p.p.m. in the form of ammonium and nitrate nitrogen, was used for making culture solution. As normal flowering period of the two varieties differed by about seven days (*Aikoku* flowering earlier than *Ptb* 10), photoperiod treatment was given to synchronise their flowering, so that samples for analysis could be taken simultaneously and almost at the same stage of growth of the two varieties. Experimental plants were subjected to a long photoperiod of sixteen hours from January 5 to February 13 and later to a short photoperiod of eight hours from February 14 till grain setting. Long photoperiod was used to keep both the varieties in the vegetative stage and later short photoperiod was given to induce flowering. As the response to short-day treatment in hastening flowering was greater in *Ptb* 10 (as studied from a separate series of trials), both varieties flowered almost within a day of each other. One set of plants was sampled for analysis at the vegetative stage on February 13, at the time of changing over from long day to short day treatment and the second sample was taken at the reproductive stage on February 28, about ten days before actual ear-emergence. Crop for yield was harvested on April 9.

Experimental Results

Plant samples collected at three stages of growth, the vegetative stage, the reproductive stage and the harvest sample respectively were analysed for top length, root length, tiller number, weight of dry matter, nitrogen content, yield, grain characters and different yield components. Results are summarised below.

(*a*) *Growth at vegetative and reproductive stages.*—Data on top length, root length, tiller number and dry matter produced at two stages of development is given in Table I.

At the vegetative stage, top length of both *indica* and *japonica* increases with nitrogen level, increase being marked from 0–20 p.p.m. Top length of *indica* is however greater than that of *japonica* at all corresponding nitrogen levels. Root length of both the varieties decreases with nitrogen level upto 20 p.p.m. and *indica* has invariably longer root than *japonica* at all corresponding nitrogen levels.

TABLE I

Comparison of growth at vegetative and reproductive stages of indica (Ptb 10) *and* japonica (Aikoku) *varieties grown at different nitrogen levels*

Variety / Levels of nitrogen p.p.m.	*Indica* (*Ptb* 10)					*Japonica* (*Aikoku*)				
	0	5	20	60	150	0	5	20	60	150
					(*i*) Vegetative stage (February 13)					
Top length cm.	47	60	63	67	67	38	45	49	50	5
Root length cm.	33	28	22	22	21	28	21	20	19	1
Tillers/Pot	9	19	21	23	23	10	22	28	30	3
Dry weight gm./Pot—										
Shoot	1·2	3·0	4·4	5·6	6·3	1·0	2·5	4·1	4·9	5·
Root	0·6	0·9	1·2	1·3	1·4	0·4	0·7	1·0	1·0	1·
					(*ii*) Reproductive stage (February 28)					
Top length cm.	62	83	99	98	95	45	60	66	71	7
Root length cm.	51	50	40	23	18	36	37	26	20	1
Tillers/Pot	9	21	38	44	51	9	28	43	58	6
Dry weight gm./Pot—										
Shoot	2·8	14·6	29·3	31·9	35·4	2·5	12·6	24·8	31·4	42·
Root	1·1	5·0	5·6	5·4	5·3	0·8	2·9	3·1	3·5	3·

Growth characters at reproductive stage also follow more or less the same pattern. At this stage, root of *indica* is very much longer than that of *japonica* at nitrogen level upto 20 p.p.m. The difference in root development of two varieties is shown in Plate III, which gives the growth condition of crop at 20 and 150 p.p.m. respectively after flowering on March 20. *Indica* at 20 p.p.m. has a vigorous root growth, whereas at 150 p.p.m., it has a poor and shallow root. *Japonica*, on the other hand, has a better root development at 150 p.p.m. than at 20 p.p.m.

Tiller number per pot for two varieties at both stages of development increases with nitrogen levels, the increase being more marked at the reproductive stage. *Japonica* variety has invariably more tillers per pot than

indica variety at all corresponding nitrogen levels. Dry weight of shoot and root of *indica* at corresponding nitrogen levels is higher than that of *japonica* at vegetative stage. Later at reproductive stage, *indica* shows higher dry weight of shoot upto 20 p.p.m. nitrogen only. At 60 p.p.m. nitrogen, both varieties show almost same dry weight of shoot but at 150 p.p.m., *japonica* surpasses *indica* in this respect.

(*b*) *Growth and yield at harvest.*—Table II and Fig. 1 (*a*) & (*b*) show the growth and dry matter of grain, straw and root at harvest.

TABLE II

Growth and yield at harvest of indica (Ptb 10) *and* japonica (Aikoku) *at different nitrogen levels*

Variety	*Indica* (*Ptb* 10)					*Japonica* (*Aikoku*)				
Levels of nitrogen p.p.m.	0	5	20	60	150	0	5	20	60	150
Top length cm.	81	98	114	109	107	49	60	70	73	73
Root length cm.	56	51	46	26	23	41	33	31	28	27
Tillers/Pot	8	21	40	56	66	10	27	43	60	66
Ear/Pot	8	18	38	54	54	4	23	42	60	62
Dry weight gm./Pot.—										
Ear	5	24	64	61	49	1·5	19	45	73	70
Straw	9	23	51	77	92	7	20	33	61	68
Root	3	8	19	17	16	1·5	5	11	12	10
Grain weight	4·2	20	58	54	45	1·0	16	40	64	62
(Grain/Straw) × 100	47	87	114	70	49	14	80	121	105	91

At harvest stage, the top length of *indica* is very much higher than that of *japonica* at all nitrogen levels. The increase in top length of *indica* between reproductive and harvest stages is highly pronounced; the *japonica*, on the other hand, shows hardly any increase in top length during this period. Ear-bearing tillers in the *japonica* variety are more than those in the *indica* variety at all levels except at no nitrogen level, where *japonica* has fewer ears per pot.

At lower nitrogen levels upto 20 p.p.m., *indica* gives more grain yield than *japonica*, while at higher nitrogen levels, *japonica* surpasses *indica* in

grain yield. From Fig. 1 (*a*), it is seen that highest grain yield of *indica* is obtained at 20 p.p.m. nitrogen, whereas *japonica* gives highest yield at 60 p.p.m., with no appreciable decrease even at 150 p.p.m. Unlike grain

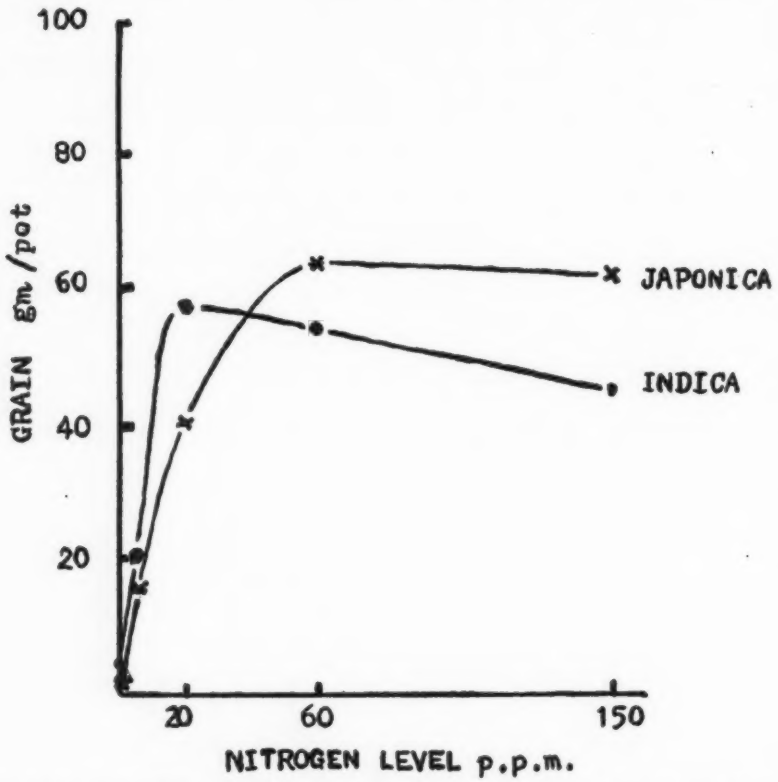

FIG. 1 (*a*). Grain yield of *indica* and *japonica* varieties grown at different nitrogen levels.

yield, straw yield continues to increase with nitrogen levels upto 150 p.p.m. in case of both the varieties.

(*c*) *Nitrogen content.*—Nitrogen content in the samples collected at vegetative and reproductive stages are shown in Table III.

Indica has generally low total nitrogen content than *japonica* at corresponding nitrogen levels upto 20 p.p.m. At higher nitrogen levels of 60 and 150 p.p.m., there is, however, no difference between nitrogen content of two varieties at vegetative stage, but at reproductive stage, *indica* has higher nitrogen content than *japonica* at both these levels. The ratio of soluble nitrogen

TABLE III

Total, soluble and protein nitrogen content of shoot of indica (Ptb 10) *and* japonica (Aikoku) *at vegetative and reproductive stages*

(Percentage on dry matter)

Variety / Levels of nitrogen p.p.m.	*Indica* (*Ptb* 10)					*Japonica* (*Aikoku*)				
	0	5	20	60	150	0	5	20	60	150
					(*i*) Vegetative stage (February 13)					
Total N	2·32	2·72	3·62	3·87	4·13	3·00	3·15	3·85	3·85	4·13
Protein N	1·85	2·07	2·56	2·79	2·81	2·50	2·41	2·80	2·83	2·80
Soluble N*	0·47	0·65	1·06	1·08	1·32	0·50	0·74	1·05	1·02	1·32
$\frac{\text{Soluble N}}{\text{Protein N}} \times 100$	25	31	41	39	47	20	31	38	36	47
					(*ii*) Reproductive stage (February 28)					
Total N	1·35	1·50	2·50	3·33	3·95	1·60	1·80	2·89	3·12	3·47
Protein N	1·06	1·12	1·87	2·33	2·55	1·27	1·35	2·19	2·31	2·39
Soluble N*	0·29	0·38	0·63	1·00	1·40	0·33	0·45	0·70	0·81	1·08
$\frac{\text{Soluble N}}{\text{Protein N}} \times 100$	27	34	34	43	55	26	33	32	35	45

* Soluble nitrogen was determined by extraction with 1% acetic acid.

to protein nitrogen, which is taken as a criterion for efficient utilization of nitrogen by plant, is also shown in Table III. At vegetative stage, there is practically no difference in this ratio of *indica* and *japonica* varieties at corresponding levels, but at the reproductive stage, this ratio at 60 and 150 p.p.m. nitrogen is higher for *indica* variety than for *japonica* variety. This shows differential character of two varieties to utilize nitrogen at the reproductive stage, where, at high level, the nitrogen metabolism of *indica* appears disturb ed due to nitrogen excess.

Nitrogen content of various parts of the plant in the harvest material is shown in Table IV and represented graphically in Fig. 2 (*a*) & (*b*).

TABLE IV

Nitrogen content at harvest of indica (Ptb 10) *and* japonica (Aikoku) *grown at different nitrogen levels*

Variety	*Indica* (*Ptb* 10)					*Japonica* (*Aikoku*)				
Levels of nitrogen p.p.m.	0	5	20	60	150	0	5	20	60	150
Ear ..	1·04	1·30	1·65	1·98	2·16	1·30	1·33	1·42	1·53	1·87
Straw ..	0·53	0·65	0·75	2·59	3·46	1·00	1·08	1·20	1·38	2·70
Root ..	0·68	0·72	0·94	2·00	2·34	1·04	1·04	1·24	1·36	2·66

TABLE V

Yield components of indica (Ptb 10) *and* japonica (Aikoku) *grown at different nitrogen levels*

Variety	*Indica* (*Ptb* 10)					*Japonica* (*Aikoku*)				
Levels of nitrogen p.p.m.	0	5	20	60	150	0	5	20	60	150
Panicle number/ plant	2·70	6·00	12·70	18·00	18·00	1·30	7·70	14·00	20·00	20·70
Panicle weight gm.	0·63	1·34	1·68	1·12	0·90	0·37	1·00	1·00	1·23	1·16
Panicle length cm.	15·60	16·00	18·30	17·80	16·90	11·00	13·30	13·80	16·40	15·10
Number of filled grain/panicle	27	48	71	50	45	10	38	41	45	47
No. of chaff/panicle	13	10	8	11	16	21	13	20	20	23
1000 grain weight gm.	19·30	22·70	22·80	21·00	19·20	20·50	23·00	23·00	22·70	21·80
Grain size mm.—										
Length	8·31	8·38	8·38	7·92	7·67	6·65	6·96	6·81	6·65	6·70
Breadth	3·12	3·07	2·90	2·82	2·67	3·07	3·30	3·12	3·20	3·20
Thickness	1·98	1·98	2·03	1·98	1·91	2·41	2·29	2·28	2·24	2·16

Nitrogen content at harvest of both varieties increases with increasing nitrogen level in the culture solution. Generally speaking, at lower nitrogen levels, *japonica* has a higher nitrogen content, whereas at higher nitrogen levels, *indica* has a higher nitrogen content. Nitrogen content of straw in

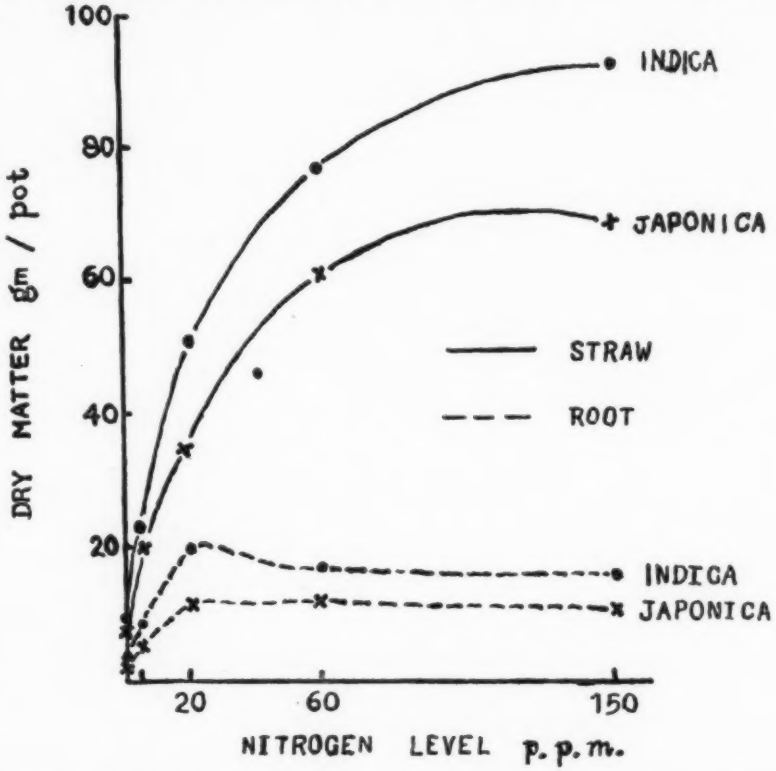

FIG. 1 (*b*). Dry weight of straw and root at harvest of *indica* and *japonica* varieties grown at different nitrogen levels.

indica increases remarkably from 20 to 60 p.p.m. nitrogen level, whereas depression in the grain yield sets in at this stage. This shows that a disturbance in the nitrogen metabolism of the variety takes place at higher nitrogen levels.

(*d*) *Yield components.*—Influence of increasing levels of nitrogen on different yield components of two varieties is shown in Table V.

Mean number of panicles per plant increases with nitrogen levels in both the varieties. *Japonica* generally has more panicles per plant than *indica*

at all nitrogen levels except when no nitrogen is given. Mean panicle weight of both varieties also increases upto a certain level and latter decreases at

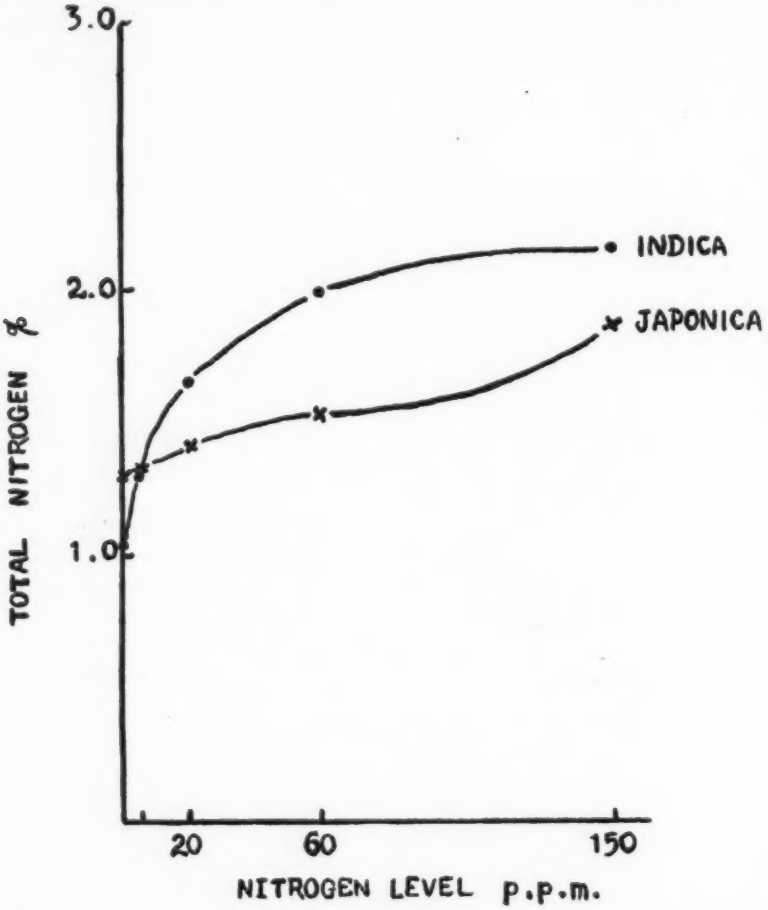

FIG. 2 (*a*). Total nitrogen content of ear of *indica* and *japonica* varieties grown at different nitrogen levels.

higher levels. Maximum panicle weight for *indica* is obtained at 20 p.p.m. while that for *japonica* is obtained at 60 p.p.m. nitrogen. At optimum nitrogen levels for panicle weight, *indica* has however a heavier panicle than *japonica*. Maximum number of filled grain per panicle for *indica* is also obtained at 20 p.p.m. nitrogen, while that for *japonica* is at 150 p.p.m. nitrogen. Maximum 1,000 grain weight for both varieties is obtained at 20 p.p.m. nitrogen,

With *indica*, the decrease in 1,000 grain weight is remarkable at 60 p.p.m. nitrogen and above. Mean length and breadth of *indica* grain generally decrease with nitrogen levels above 20 p.p.m., while there is no appreciable difference in the thickness of the grain of this variety with increase in nitrogen

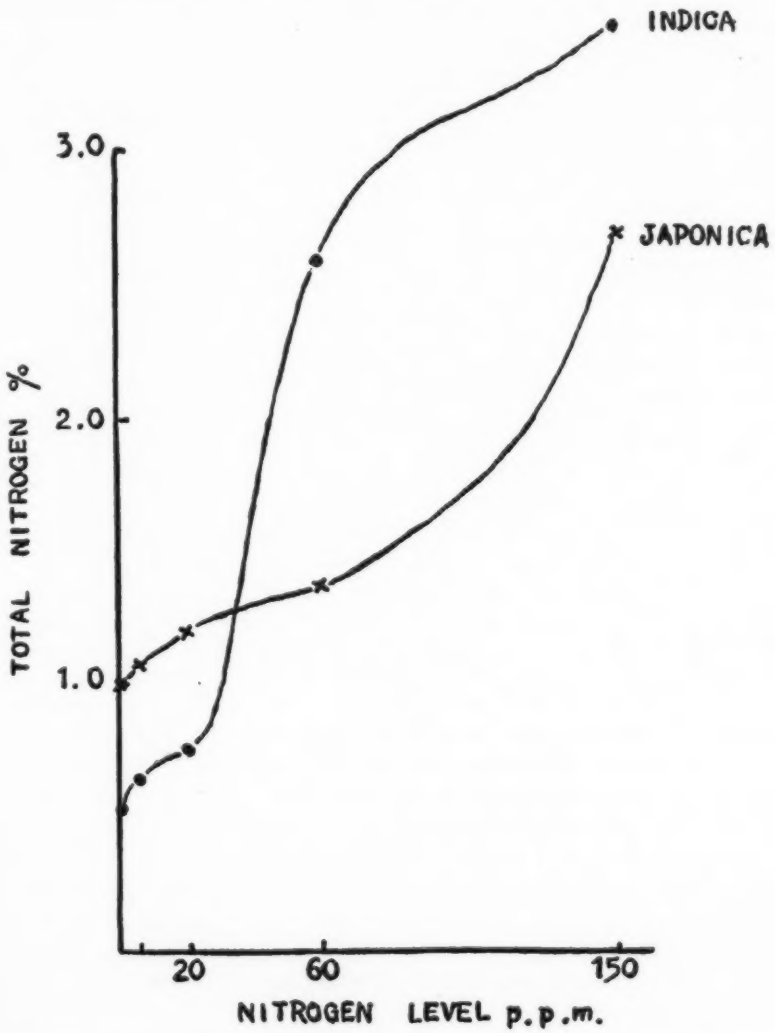

FIG. 2 (*b*). Total nitrogen content of straw of *indica* and *japonica* varieties grown at different nitrogen levels.

supply. With *japonica* variety also, there is decrease in the mean length and breadth of the grain with increasing nitrogen levels above 5 p.p.m., but the decrease is not so pronounced as in the case of *indica*. Mean thickness of grain of *japonica* however decreases with increase in nitrogen supply.

DISCUSSION

Results presented show the differential behaviour of *indica* and *japonica* varieties of rice under the influence of increasing levels of nitrogen. Growth differentiation between the two varieties are marked at all stages of development. *Japonica* variety is shorter than *indica* at all nitrogen levels but has a capacity to produce more tillers per plant than the latter. At low nitrogen supply upto 20 p.p.m., *indica* gives more grain yield than *japonica*, but at higher nitrogen levels, *japonica* shows more grain yield. Unlike grain yield, straw yield of both varieties continues to increase with increase in nitrogen supply, *indica* always giving a higher yield of straw than *japonica* at corresponding nitrogen levels. It is thus seen that with both varieties, straw yield, which is the result of vegetative growth, can be pushed up effectively by heavy nitrogen application, while grain yield, a result of reproductive growth, attains its maximum value only at specific nitrogen levels for each variety, 20 p.p.m. for *indica* and 60 p.p.m. for *japonica*. At higher levels of nitrogen, where *japonica* continues to respond effectively in grain yield, *indica* shows a depression in yield. The root development of *indica* was also impaired at nitrogen levels of 60 p.p.m. and above, while *japonica* showed a healthy root growth right upto 150 p.p.m. nitrogen (Plate III).

Utilization of nitrogen for production of dry matter by the two varieties is shown by the ratio of soluble to protein nitrogen given in Table III. Baba (*l.c.*), reporting on " Breeding of Rice Varieties Suitable for Heavy Manuring " has characterised this ratio as an indication of utilization of absorbed nitrogen in the production of grain. Comparing the suitability of *indica* and *japonica* varieties for adaptability to heavy manuring conditions, he has observed that *indica* varieties, when put under high manuring levels, accumulate more soluble nitrogen at early stages of development ,resulting in higher ratio of soluble to protein nitrogen in the plant body as compared to *japonica* varieties. This early accumulation of excess soluble nitrogen in *indica* suppresses further absorption of nitrogen at latter stages, leading to unbalanced nitrogen nutrition. Early excessive increase in soluble nitrogen in *indica* at high manuring levels causes increase in leaf area and dry matter and the latter suppressed absorption, presumably because of decreased absorption activity of the roots, results in low grain yield and high yield of straw. The *japonica* varieties on the other hand, utilise nitrogen efficiently at high levels

of manuring, the protein content keeping pace with the absorbed nitrogen and the ratio of soluble to protein nitrogen is comparatively low.

In the present study, the ratio of soluble to protein nitrogen has been measured both at vegetative and reproductive stages. At vegetative stage, no difference in the ratio of soluble to protein nitrogen is observed between the two varieties at corresponding nitrogen levels. At reproductive stage however, this ratio for *indica* is very much higher at 60 and 150 p.p.m. nitrogen, showing that while at vegetative stage, there is no difference between nitrogen metabolism of the two varieties, at reproductive stage, *indica*, at higher nitrogen levels of 60 and 150 p.p.m., is not able to utilize absorbed nitrogen as efficiently as *japonica*. This low efficiency of *indica* at high nitrogen levels decreases grain yield, increases straw yield and grain to straw ratio decreases rapidly after 20 p.p.m. nitrogen level (Table II). *Japonica* variety on the other hand utilising nitrogen efficiently at high nitrogen levels, shows increased grain yield upto 60 p.p.m. and there is no appreciable decrease in grain yield and grain to straw ratio even upto 150 p.p.m. nitrogen. *Japonica* variety, both at vegetative and reproductive stages, has higher total nitrogen content upto a level of 20 p.p.m. nitrogen. Later at reproductive stage, *indica* shows higher nitrogen content at 60 and 150 p.p.m. nitrogen. In the harvest sample also (Table IV), nitrogen content in straw of *indica* is very much higher than that of *japonica* at 60 and 150 p.p.m. nitrogen. This shows that in *indica* variety, at lower nitrogen levels, nitrogen is efficiently translocated to the ear and utilised in grain formation, while at high nitrogen levels, this translocation is suppressed with the consequent increase in the nitrogen content of straw at harvest. In the *japonica* variety, translocation of nitrogen from vegetative to reproductive parts at higher nitrogen levels appears to be efficient and results in higher yield of grain.

Grain yield of *japonica* variety at nitrogen level of 20 p.p.m. and less is poorer than that of *indica*. The *japonica* variety has, therefore, a higher nitrogen requirement for optimum yield than *indica*. Tanaka *et al.* (*l.c.*) have earlier observed that nitrogen requirement of *indica* (*Ptb* 10 grown during the main crop season) for optimum yield lies between 20 and 40 p.p.m. nitrogen. In the present series of trials, nitrogen requirement of the same variety grown during the second crop season is seen to be 20 p.p.m. nitrogen (*plus* about 3 p.p.m. supplied in water used for making culture solution). *Japonica* variety on the other hand, is found to need 60 p.p.m. for optimum yield and there is no appreciable decrease even at 150 p.p.m.

The *indica* variety at nitrogen levels over 20 p.p.m. shows decrease in grain yield, although mean number of panicles per plant is more at higher

nitrogen levels. There is however a decrease in the mean panicle weight with heavy manuring, because of less number of filled grains per panicle and decrease in 1,000 grain weight. The depressed yield of *indica* at higher nitrogen levels is therefore not due to decrease in ear-bearing tillers but due to decrease in number and weight of set grains per panicle. *Japonica* variety on the other hand gives higher grain yield with heavy manuring. Mean number of panicles per plant continues to increase and the panicle weight is kept rather constant. Number of filled grain per panicle is more at higher nitrogen levels but there is a slight depression in 1,000 grain weight. These results also show that the depression in the yield of grain of *indica* at heavy manuring is mainly caused at the reproductive stage and not earlier at vegetative stage. *Japonica* variety on the other hand suffers no such ill-effects with heavy manuring.

Comparing the two varieties at their optimum nitrogen levels of 20 and 60 p.p.m. respectively, it is seen that the *indica* variety has a heavier and longer panicle than the *japonica* variety. The latter, on the other hand, has considerably more ear-bearing tillers. The *indica* variety is therefore classed as the " panicle weight " type and the *japonica* as the " panicle number " type. This classification based on various morphological characters has been adopted by various Japanese workers (Baba, *l.c.*) to distinguish varieties that can stand heavy manuring. They suggested that the varieties of " panicle weight " type are suited for light manuring only and when put under heavy manuring conditions, lodge badly and give poor yield performance, a character usually associated with *indica* varieties. The " panicle number" type varieties on the other hand are suited for heavy manuring, they have shorter length and are strongly resistant to lodging and give good yield performances only at high degree of manuring. At low levels of manuring however, these latter varieties fare badly and give poor harvest.

SUMMARY

Comparative efficiency of a short duration *indica* variety (*Ptb* 10) and a *japonica* variety (*Aikoku*) has been tested under increasing nitrogen levels in water culture trials. The *indica* variety does well at 20 p.p.m. of nitrogen, but at higher nitrogen levels the grain yield is pulled down. The *japonica* variety on the other hand grows well at nitrogen levels of 60 p.p.m. and there is no appreciable decrease in grain yield even at 150 p.p.m. nitrogen. The *japonica* variety however fares very badly at nitrogen levels of 20 p.p.m. and less and the grain yield at these levels is even poorer than that of the *indica* variety.

FIG. 1. Growth condition after flowering of *indica* and *japonica* varieties at 20 and 150 p.p.m. nitrogen.

Efficiency of nitrogen utilization by two varieties has been measured by the ratio of soluble to protein nitrogen in the plant both at vegetative and reproductive stages. There is no difference in this ratio of the two varieties at the vegetative stage. At the reproductive stage however, the *indica* variety has a higher ratio of soluble to protein nitrogen both at 60 and 150 p.p.m. nitrogen than that of the *japonica* variety, showing a disturbed nitrogen metabolism of *indica* under high manuring condition. The depression of the grain yield of *indica* at higher nitrogen levels is also seen to be due to decrease both in the number of filled grains per panicle and weight of 1,000 grains, factors which are mainly decided at the reproductive stage. It is therefore concluded that the nitrogen metabolism of *indica* gets disturbed at the reproductive stage due to excess nitrogen level and the variety is not able to utilise nitrogen efficiently for formation of grain. The *japonica* variety however, utilises nitrogen efficiently under high manuring conditions and gives greater yield.

The authors are indebted to Shri R. L. M. Ghose, Director, Central Rice Research Institute, Cuttack, for suggestions and improvement in the text of the paper.

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CARBON-NITROGEN METABOLISM OF SOIL FUNGI

V. Role of Cyanide, Nitrite and Sulphur

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Introduction

The ability of Fusaria to metabolise various carbon and nitrogen sources and grow in media of varying hydrogen-ion concentration has been shown in earlier parts (Natarajan, 1956, 1958). Since both nitrate nitrogen and ammonia nitrogen end up as amino nitrogen in cell proteins, reduction is an essential step. It was expected that by the use of potassium cyanide, potassium nitrite and elementary sulphur, the growth of *Fusarium* species would indicate the intermediate stages in the nitrogen utilization. These studies are presented in this communication.

Materials and Methods

The culture of the organisms used in this work was obtained from Centralbureau Voor Schimmelcultures (Baarn). *Fusarium vasinfectum* and *Fusarium udum* were grown in Richard's nutrient solution (Rawlins, 1933). The carbon sources were varied by the use of different monosaccharides and disaccharides. With each carbon source, sodium nitrate was used as the nitrogen source. Equivalent quantities of carbon and nitrogen sources were used at the optimum C: N ratio reported in the earlier parts (Natarajan, 1956). The cultural methods employed were similar to those previously described (Natarajan, 1958). Nitrite was detected by the colour reaction of Griess as modified by Blom (1926). Hydrogen sulphide was detected with lead acetate paper strips. Ammonia was detected by Nessler's reagent.

The two Fusaria were grown separately under the specified conditions. The culture filtrate was analysed for nitrite and ammonia in each case. The fungi were grown with and without the addition of potassium nitrite, the total nitrogen concentration being kept at 70 mg./50 ml. to study the effect of potassium nitrite; the mat weight and nitrogen in the mat were determined

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TABLE I

Toxicity of nitrite on Fusaria in presence of various carbon sources on 20th day of incubation

The sum of nitrite nitrogen and nitrate nitrogen is 70 mg. per 50 ml. medium and the amount of nitrite nitrogen is indicated in the table and the balance is nitrate nitrogen.

Carbon source	Nitrite nitrogen/ 50 ml. medium (mg.)	*F. vasinfectum*		*F. udum*	
		Mat weight (mg.)	% nitrogen in mat	Mat weight (mg.)	% nitrogen in mat
Glucose	0	350·9	5·096	314·7	4·700
	1	339·2	4·851	300·0	4·512
	2	330·0	4·561	294·0	4·316
Fructose	0	600·6	6·135	420·9	5·406
	1	590·2	5·946	411·2	5·200
	2	580·6	5·528	400·0	5·000
Mannose	0	335·2	4·833	306·2	4·601
	1	320·0	4·612	300·0	4·315
	2	314·2	4·412	280·1	4·160
Galactose	0	264·0	4·000	180·2	3·546
	1	250·0	3·861	171·2	3·220
	2	241·9	3·521	166·2	3·000
Sucrose	0	565·4	5·499	360·8	5·211
	1	550·2	5·212	340·0	5·000
	2	540·3	5·015	330·2	4·815
Maltose	0	370·9	5·299	320·6	4·842
	1	360·1	5·012	310·0	4·612
	2	345·2	4·812	300·2	4·426
Lactose	0	280·2	4·554	190·5	4·399
	1	265·0	4·315	180·2	4·185
	2	250·0	4·156	171·5	3·912

on the 20th day of incubation. This was repeated using potassium cyanide in place of nitrite at a concentration of 4 mg./50 ml. and mat weight determined on the 9th day of incubation. The fungi were also grown with the addition of different concentrations (1 and 2 mg./50 ml.) of elementary sulphur, a control also being studied at the same time. The mat weight was noted on the 8th and 16th day of incubation in this series.

DATA AND DISCUSSION

When sodium nitrate was used as the nitrogen source with the carbon sources, fructose, sucrose, maltose, mannose, glucose, galactose, lactose, nitrites could be detected in culture filtrate in small quantities, but the greater part was found to be ammonia nitrogen. When 20-day old mats developed on these media were incubated with 1% alkaline sodium nitrate, again nitrites were detected. This shows *F. vasinfectum* and *F. udum* reduced nitrates to nitrites. Two consecutive reactions may be visualised in the system.

$$\text{Nitrite} \xrightarrow{k_2} \text{Ammonia} \qquad (1)$$

$$\text{Nitrates} \xrightarrow{k_1} \text{Nitrites} \qquad (2)$$

The small concentrations of nitrite coupled with the toxicity of nitrites in larger amounts (Table I) suggest that the reaction (1) is faster than (2). This indicates the presence of a nitrate reductase in the two Fusaria studied. Nitrates may also function as hydrogen acceptors in the presence of the dehydrogenases in Fusaria (Nord and Mull, 1945). In the presence of potassium cyanide our observations showed that the growth of *F. vasinfectum*

TABLE II

The influence of added potassium cyanide on Fusaria in presence of various carbon sources on the 8th day of incubation

4 mg. KCN is added after 4 days growth

Carbon source	*F. vasinfectum*		*F. udum*	
	Mat weight (mg.) without KCN	Mat weight (mg.) with KCN	Mat weight (mg.) without KCN	Mat weight (mg.) with KCN
Glucose	260·2	261·0	180·7	181·1
Fructose	320·2	319·2	240·9	241·9
Mannose	250·2	248·9	165·8	164·7
Galactose	135·2	136·1	94·3	95·0
Sucrose	284·6	285·1	218·6	219·0
Maltose	275·2	274·8	190·4	190·0
Lactose	140·4	141·1	100·2	100·5

and *F. udum* was unaffected by the potassium cyanide in the concentrations used (Table II). Apparently a potassium cyanide insensitive 'Nitrate reductase' is present. Nitrite was detected under these conditions too. When the fungi were grown in presence of nitrite a marked decrease in mat weight and percent nitrogen in mat were noticed (Table I). The addition of elementary sulphur led to positive tests for hydrogen sulphide during growth of both Fusaria indicating that sulphur acts as a hydrogen acceptor in the medium used and no nitrite could be detected in the culture. The growth is better, as shown by mat weight (Table III). It is therefore possible

TABLE III

The influence of added sulphur on Fusaria in presence of various carbon sources

Carbon source	Sulphur added/50 ml. medium (mg.)	Mat weight (mg.)			
		F. vasinfectum		*F. udum*	
		Incubation		Incubation	
		8 days	16 days	8 days	16 days
Fructose ..	0	320·2	560·8	240·9	390·8
	1	326·8	570·1	248·2	400·1
	2	330·1	580·1	260·1	408·2
Sucrose ..	0	284·6	525·2	218·6	320·4
	1	290·1	535·1	226·1	330·2
	2	300·1	548·1	230·2	340·1
Maltose ..	0	275·2	355·2	190·4	295·4
	1	280·7	360·7	200·2	305·8
	2	285·2	364·5	208·3	309·1
Glucose ..	0	260·9	331·4	188·7	280·8
	1	265·1	340·2	190·2	290·3
	2	270·2	348·2	196·7	296·4
Mannose ..	0	250·2	320·5	165·8	275·4
	1	260·2	328·2	172·4	286·8
	2	265·6	334·2	181·4	290·4
Lactose ..	0	140·4	248·4	100·2	165·2
	1	146·8	259·1	108·6	170·3
	2	150·2	264·3	112·3	180·2
Galactose ..	0	135·3	240·1	94·3	161·3
	1	140·1	251·2	98·4	166·6
	2	146·2	260·1	108·2	172·4

that the apparent absence of nitrites in the presence of sulphur is due to its functioning as an oxidizing agent towards hydrogen sulphide. In other words the reaction between elementary sulphur and dehydrogenases in Fusaria can be represented by the following equations:

$$S^{\circ} + 2H = H_2S$$

$$2H_2S + O_2 = 2S + 2H_2O$$

$$2H_2O + H_2S + 4O = SO_4^{=} + 2H_3O^{+}.$$

SUMMARY

Fusaria reduce nitrates to nitrite and ammonia irrespective of the carbon source. Fusaria possess potassium cyanide insensitive nitrate reductase.

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THE CYTO-ARCHITECTURE OF THE YEAST NUCLEUS

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Introduction

The literature is replete with such statements as (1) ordinary as well as dark ground illumination are unsuitable for a study of the nucleus of living yeast (Müller, 1956); (2) it is visible under phase contrast only as an area of lesser density (Mundkur, 1954); (3) under phase as well as the ultra-violet microscope it appears optically empty and bereft of any structural differentiation (Mundkur, 1954); (4) when the refractive index of the medium is suitably adjusted, structural differentiation could be observed under phase contrast in the nucleus of some species of yeasts (Müller, 1956); (5) the diameter of the resting nucleus is only *ca* $0{\cdot}5\,\mu$ and consequently there is little possibility of studying the yeast chromosomes (Winge and Roberts, 1954 *b*); (6) the body identified as the nucleus is only the ' spindle with chromatin ' and (7) it is not extra-vacuolar as generally imagined but it occurs really inside the vacuole (Lindegren, Williams and McClary, 1956).

Viewed in a historical perspective these divergences of opinion reflecting broadly, as they do, the earlier disagreements (see Wager, 1898) would imply that there has been no real advance in the field during the past several decades. Though Nägeli saw the nucleus in a living yeast in 1844, it was only in 1896 that Henneguy confirmed its rare visibility in the living condition.

The opinion of the investigators during the years that intervened was rather varied. Some were sceptical about its very existence. Schmitz (1879), Strasburger (1887), Hansen (1896) and Moeller (1893) revealed it as a homogeneous body by fixatives and stains (see Wager, 1898), while Dangeard (1892) offered evidence that it was vesicular having a nuclear membrane and a ' nucleolus '.

The real advance over Dangeard was the use by Henneguy (1896) of the living nucleus as a standard. He saw the nucleus in a living red yeast, compared its structure with that seen in fixed and stained preparations and

confirmed Dangeard's observation that it has a nuclear membrane and a 'nucleolus'. The vesicular nature of the nucleus was emphasized by Guilliermond (1920). Subramaniam (1946) and Lietz (1951) confirmed the presence of a nuclear membrane from stained preparations. The similarity in the divergence of views, now as in the past, necessitates an explanation.

OBSERVATIONS

A. Conditions under which the Nucleus is Visible in Living Cells

In *Saccharomyces cerevisiæ*, NRRL, Y-567, the resting nucleus is clearly visible in wort cultures only from the fourth day onwards after inoculation (Royan and Subramaniam, 1956; Royan, 1956 *a*, *b*, *c*; 1958). Thus aging as well as starving the cells is necessary to render the nucleus visible. During this process of aging, glycogen gradually disappears from the cytoplasm and there is also a decrease in the cytoplasmic basophilia.

If the intense cytoplasmic basophilia as well as the abundant presence of glycogen are responsible for the invisibility of the nucleus in young cells, then, mere adjustment of the refractive index of the medium (Müller, 1956) may not reveal the real structure of the nucleus. It may appear only as a homogeneous area exhibiting no structural differentiation (Mundkur, 1954). In cells from 5–7 day cultures, a phase contrast microscope is not imperative for locating the nucleus.

B. Structure of the Nucleus under Ordinary as well as Dark Ground Illumination

For photographing the living nucleus under ordinary illumination it is necessary to choose cells in which it exhibits a sharp contrast from the cytoplasm. Photos 1 and 2 illustrate its structure as well as orientation in relation to the vacuole and confirm the possibility that such a nucleus may have been the basis for Henneguy's (1896) report. The vesicular nucleus has a formed structure inside and is delimited from the cytoplasm by a nuclear membrane. Even in plants, where it is generally believed that the nucleus has only a phase boundary, the nuclear membrane appeared well defined when the root-tip cells of *Allium cepa* were stained with Giemsa (Subramanyam and Subramaniam, 1957).

Evidence was adduced earlier (Royan, 1956 *b*) that in favourable examples the nucleus was visible under the ultra-microscope. Photos 2 and 3 are of the same cell under ordinary as well as dark ground illumination. Observation under the ultra-microscope is complicated by the particles lying near the nucleus reflecting light and thus masking its structure as in Photo 3

(compare with Photo 2). The nuclear membrane is clear near the vacuole (Photo 3). Examples like the cell in Photo 8 are, however, available in which the nuclear membrane is clear except in the region obscured by a pair of grains.

C. Formed Structures Inside the Nucleus

Henneguy (1896) as well as Guilliermond (1920) refer to the presence of a nucleolus within the nucleus. While the formed structures inside the nucleus in micrographs 1 and 2 are reminiscent of the nucleolus of higher organisms, they represent only one, or probably two, of the variations usually observed. Examination of living cells under phase contrast (Photo 4) reveals also instances in which two rod-shaped bodies are seen inside the area bounded by the nuclear membrane.

All these structures come under the general category of nucleoli. Since 1892 (Sharp, 1934) the nucleoli of higher organisms have been separated into two distinct types, *viz.*, (i) *karyosomes* or chromatin nucleoli and (ii) *plasmosomes* or plastin nucleoli. With the discovery of the 'nukleal reaktion' by Feulgen, the use of the term 'nucleolus' has been restricted to the so-called plastin nucleolus of earlier workers and the term *chromocenter* has been substituted for the karyosome.

When smears of a five-day culture fixed in iodine-formol-acetic solution and hydrolysed at 60° C. for 9 min., are stained in leuco basic fuchsin for 90 min., only a portion of the nucleus (Photos 5, 6 and 7) is stained (Thyagarajan and Subramaniam, 1957; Royan, 1958). A comparison of Photo 5 with Photos 1 and 2 would suggest that the formed body inside the nucleus is not the nucleolus but is the chromocenter.

The interesting condition is the presence of a pair of rods within the nuclear membrane (Photo 4). The relative rarity of such stages and unsolved technical difficulties precluded following up the same cell during fixation, hydrolysis and staining. Comparable stages are present in Feulgen preparations (Photos 6 and 7). The nuclear membrane is not clear in Photos 6 and 7 taken under ordinary illumination, but the pair of Feulgen positive rods are seen to occupy only a portion of the nucleus. Examination of the same cells under phase contrast confirm (1) the existence of the nuclear membrane and (2) that the rods constitute only a portion of the nucleus.

D. Evidence for the Extra-Vacuolar Position of the Nucleus

A perusal of micrographs 1–4 would indicate that the well-defined nuclear membrane delimits the nucleus not only from the cytoplasm but also from the vacuole. Under dark ground illumination the luminous

nuclear membrane could be seen in favourable examples (Photo 8). A comparison of ultra-micrographs 3 and 8 with light (Photos 1 and 2) and phase pictures (Photo 4) would offer proof that the nuclear membrane could be seen in all the three types of illumination.

Evidence was adduced earlier from studies with the ultra-microscope (Royan, 1956 *b*) that a membrane separates the vacuole from the cytoplasm as well as the nucleus. The extra-vacuolar position of the nucleus is exemplified by Photo 9 where the nuclear and vacuolar membranes are luminous. Because the nucleus and the vacuole are not in the same focus, the vacuolar membrane is not as crisp as the outline of the nucleus. In cells stained with hæmatoxylin (Photo 10) the extra-vacuolar position of the nucleus could only be surmised because the vacuolar membrane cannot be demonstrated. But in rare instances (Photo 11) the vacuolar membrane separates from the cytoplasm at some as yet undetermined stage during dehydration and clearing. The clear intact separation of the vacuolar membrane as seen in Photo 11 emphasises that the nucleus is an extra-vacuolar structure.

E. Identification of Bi-nuclearity

Bi-nuclearity has been reported in spores as well as zygotes. Guilliermond (1910) described the budding of bi-nucleate zygotes in the yeast, *Johannisberg* II. He records that it is often impossible to distinguish the individuality of the two nuclei owing to the difficulty in staining the nuclear membrane. The criteria used by Guilliermond to identify bi-nucleate stages are: (i) staining the yeast cells with hæmatoxylin and (ii) the presence of a nuclear membrane.

The recent description of supernumerary mitoses and bi-nucleate spores (Winge and Roberts, 1954 *a*) do not satisfy the above criteria. Azure I is said to colour the same cellular structures as Feulgen (Winge and Roberts, 1954 *a*, p. 319). When as shown in Photos 5, 6 and 7 of this paper Feulgen tints only a portion of the nucleus (Thyagarajan and Subramaniam, 1957; Royan, 1958) we have to presume that the same is the case with Azure I also. It is this *stainable portion of the nucleus* without any demonstration of a nuclear membrane that is *identified as the whole nucleus*. In the above context it is difficult to understand how they distinguished chromosomes from nuclei in their Photos 9 and 10 (Winge and Roberts, 1954 *a*, p. 320 and Pl. I).

Hæmatoxylin reveals more details since it colours both the Feulgen positive and the Feulgen negative structures lying within the area bounded by

the stained nuclear membrane (Royan, 1956 *a*). The intense contrast between the nucleus and the cytoplasm renders difficult a demonstration in the same micrograph (*a*) the structure of the nuclei as well as (*b*) their orientation in relation to the other cell organelles. Micrograph 12 shows the orientation of the two discrete nuclei in a vegetative cell and Photo 13 shows the structure of these nuclei. The nuclear membrane could be seen clearly near the unstained area of the nucleus. A comparison of Photos 12 and 13 with Photos 4, 6 and 7 would enable a clear distinction of a pair of bodies lying within the nucleus from a bi-nucleate stage.

Discussion

The conclusions that may be drawn on the basis of the evidences adduced in this paper are at variance with the views of some investigators referred to in the Introduction. (1) Contrary to Müller's experience the living nucleus could be photographed under ordinary (Photos 1 and 2), phase contrast (Photo 4) as well as dark ground illumination (Photos 3, 8 and 9). (2) Unlike Mundkur's (1954) observations the nucleus exhibits structural differentiation (Photos 1, 2 and 4–7). (3) It is not enclosed by the vacuolar membrane as claimed by Lindegren, Williams and McClary (1956) but is an extravacuolar structure (Photos 1–4, 8–11). (4) The nucleus is not small in size (compare the size of the nucleus with micrometer scale in Photos 2, 4 and 5) as claimed by Winge and Roberts (1954 *a*, *b*) because a comparison of Feulgen preparations (Photos 5–7) with living nuclei (Photos 1–4, 8 and 9) reveals that Schiff's reagent colours only a portion of the nucleus in hydrolysed cells. It is this region of the nucleus that has been identified as the whole nucleus and made the basis for their estimate of the small size of the yeast nucleus. (5) Contrary to the contention of Winge and Roberts (1954 *a*, *b*) it is possible to study the intra-nuclear structures (Photos 5, 6 and 7) and distinguish a pair of rods lying inside the nucleus from a pair of adjacent nuclei (Photos 12 and 13).

The nucleus exhibits a range of variation in structure (Photos 1–4; see also Royan, 1956 *a*). The formed structure within the membrane of the living nucleus in Photos 1 and 2 is reminiscent of the ' nucleolus ' reported by Henneguy (1896) as well as Guilliermond (1920). A corresponding body is often seen in cells stained by the Feulgen technique (Photo 5). It is therefore not a *nucleolus* but a *chromocenter*.

Occasionally the chromocenters have the shape of a pair of rods (Photos 4, 6 and 7). Subramaniam (1946) illustrated such a stage (his Fig. 18; p. 146) from Carnoy iron-hæmatoxylin preparations and described them as

two chromosomes. There are reports of a pair of chromosomes in yeasts (Badian, 1937; Robinow, 1944). Lindegren's (1946) early record of the presence of a pair of Feulgen positive rods in the so-called " centrosome " probably belongs to the same category. Being Feulgen positive (Photos 6 and 7) it has to be presumed that these two rods represent regions of chromosomes or perhaps even entire chromosomes since there are extant reports on intra-nuclear mitosis in yeasts (Lietz, 1951).

The structure of the resting nucleus revealed by this study offers the hope that the behaviour of the nucleus during budding may be capable of an orthodox analysis to elucidate whether the chromocentral rods represent whole chromosomes and whether they constitute the entire chromosome complement of the organism.

SUMMARY

1. A well-defined nuclear membrane enclosing formed structures could be observed in the living cells of *Saccharomyces cerevisiæ* (NRRL, Y-567) from 120–168 hr. wort cultures, under ordinary, phase contrast and dark ground illumination.

2. The formed structures within the nuclei correspond to the chromocenters and not the nucleoli of higher organisms.

3. A comparison of Feulgen and Hæmatoxylin stained preparations renders possible the differentiation of a pair of bodies lying within a nuclear membrane from a bi-nucleate stage.

4. Iron hæmatoxylin preparations in which the vacuolar membrane has separated from the cytoplasm confirm the extra-vacuolar position of the nucleus.

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Description of Photos

Photos 1–4, 8 and 9. Living Cells.

Photo 1. Ordinary Illumination, × *ca.* 4,200. The nuclear membrane delimits the nucleus from the cytoplasm and vacuole and encloses a formed structure.

Photos 2 and 3. Same cell under ordinary and dark ground illumination. The grains lying near the nucleus in Photo 3 mask the nuclear membrane. Photo 2, × *ca.* 4,500. Photo 3, × *ca.* 5,300.

Photo 4. Phase Contrast. × *ca.* 5,300. The nucleus contains a pair of rods.

Photos 5, 6 and 7. Iodine-formol-acetic—Feulgen. Ordinary illumination. × *ca.* 6,900. The chromocenters occupy only a portion of the nucleus. Note the pair of rods in Figs. 6 and 7.

Photos 8 and 9. Dark Ground Illumination. Photo 8. × *ca.* 5,000. The nucleus is bounded by the nuclear membrane. Photo 9. × *ca.* 5,500. The

nucleus and vacuole are separated from each other by their luminous membranes.

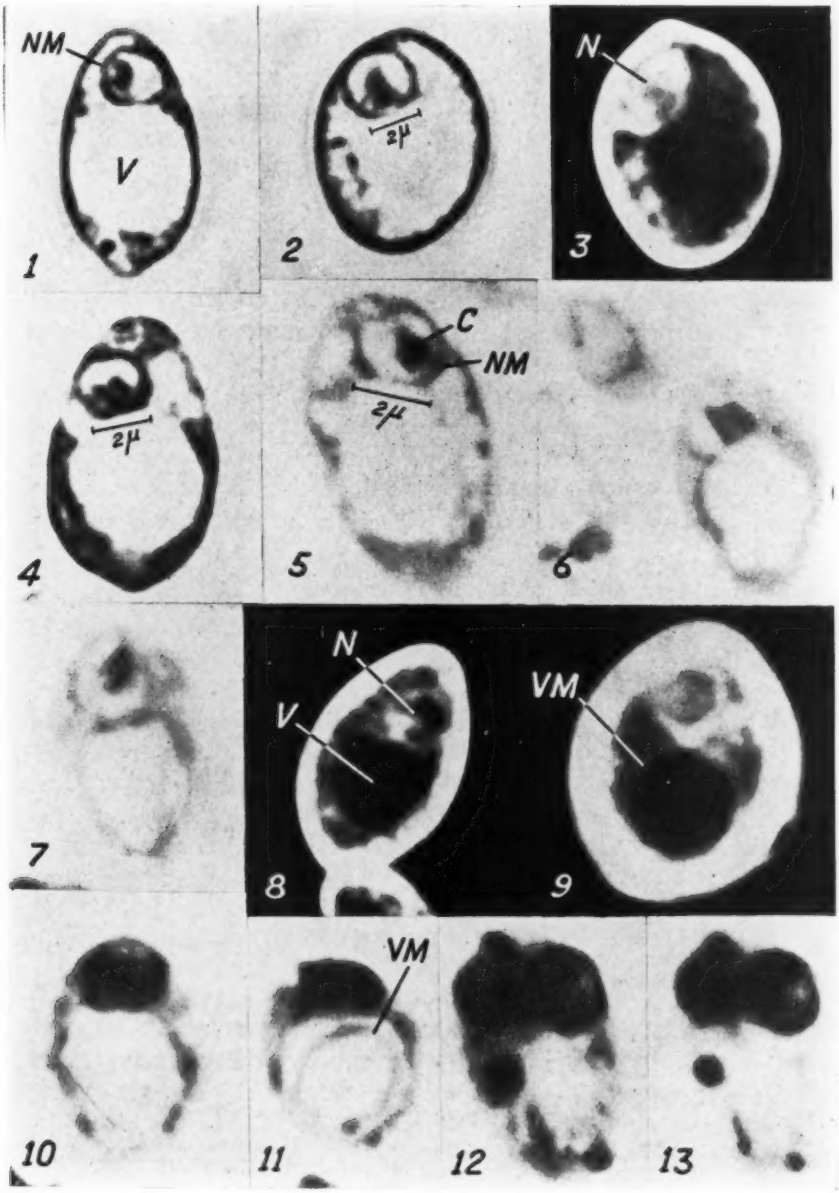
PHOTOS 10–13. Iodine-formol-acetic. Unhydrolysed. Heidenhain's iron hæmatoxylins. Ordinary illumination. × *ca.* 6,900.

PHOTO 10. Shows the orientation of the nucleus in relation to the vacuole.

PHOTO 11. The vacuolar membrane has separated from the cytoplasm. The nucleus lies outside the vacuolar membrane.

PHOTOS 12 and 13. A bi-nucleate cell in two foci.

C. Chromocenter; N. Nucleus; NM. Nuclear Membrane; V. Vacuole; VM. Vacuolar Membrane.



FIGS. 1–13

[The Plate has been reduced to Nine-tenths of its original size]

ELECTRICAL *VERSUS* CHEMICAL THEORY OF NEUROMUSCULAR TRANSMISSION

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Unstriated muscle has some interesting properties that the mechanical response produced by electric current differs in some respects from that produced by a chemical substance. These differences are most striking in *Mytilus* muscle (Singh, 1938 *a*). Substances, which produce tonic contraction, decrease the excitability to electric current and increase that to chemical stimulation, such as by potassium, acetylcholine, sodium, barium, etc. These substances are monovalent cations, lithium, sodium, ammonium, potassium and hydrogen-ions; monovalent anions, bromide, nitrate, iodide, thiocyanate; divalent cations, calcium, strontium and barium; drugs, adrenaline, acetylcholine, veratrine, caffeine, strychnine, curare, nicotine, novocaine, ephedrine, ether, chloral hydrate, chloroform.

The above factor therefore affects the excitability to electrical and chemical stimulation in opposite directions. There is another factor that affects the two excitabilities in the same direction. This factor is that of accommodation (Singh, 1938 *b*). Substances, which diminish accommodation, increase the excitability; the above substances, which produce tonic contraction, have this effect in small concentrations; if the muscle is not much sensitive to chemical stimulation, then larger concentrations increase the excitability to both chemical and electrical stimulation. Calcium increases accommodation, hence decreases excitability to both when increased beyond a certain minimum concentration, but by virtue of its tone-producing properties, it may affect the two excitabilities in the opposite directions; the minimum concentration of calcium referred to being presumably required to preserve the membrane and other structures of the muscle. Substances, which antagonise the action of calcium, will thus decrease accommodation. Large concentrations of ammonium and potassium antagonise the action of calcium, though small concentrations produce similar effects. Sudden increase in osmotic pressure of the saline by addition of sucrose or sodium chloride produces contraction (Singh, 1942), but in excess of ammonium or potassium, slow increase of osmotic pressure will produce contraction (Singh, 1939; Singh and Acharya, 1957). Toxic concentrations

decrease the excitability both to electrical as well as chemical stimulation.

In frog's stomach muscle, most of the substances mentioned above affect the two excitabilities in the same direction (Singh, 1939), but in dog's stomach muscle, though most of the substances affect the two excitabilities similarly, some of them such as excess of calcium and hydrogen-ions, which increase tone, affect the two excitabilities in opposite directions (Singh, 1940). These substances increase the response to chemical stimulation, so long as they are in the process of producing tonic contraction, that is, so long as they act as subliminal stimuli capable of potentiating and stimulating action of other tone-producing substances, but once the tonic contraction has developed and the base line raised, then the response to all forms of stimulation decreases. This is presumably due to altered properties of the contractile mechanism. It is probable that when one kind of tone develops, then the folded polypeptide chains are held together in that condition by development of cross-linkages, which then retard contraction as well as relaxation (Singh and Singh, 1954).

The above differences between the responses to chemical and electrical stimulation can, therefore, be used to decide whether nerve stimulates the muscle by virtue of its action potential or by secreting a chemical substance such as acetylcholine (Singh, 1957). Experimental findings suggest that neuromuscular transmission in unstriated muscle is an electrochemical event, the primary response being produced by the action potential, and this is tonically maintained by the secretion of acetylcholine (Singh and Singh, 1947).

In the present research, the effect of some substances which can be used to differentiate between the two excitabilities, has been studied on vagus-muscle preparation from dog's stomach.

METHODS

Nerve-muscle preparations from dog's stomach were employed by cutting out a piece of the muscle along with the vagus nerve attached (Narayana and Singh, 1944). Simultaneously two pieces from the same stomach were stimulated by alternating current (12 volts for 10 sec.), acetylcholine (1 in 10^5) or potassium (20 p.c. of the sodium of the saline being replaced with potassium). At 37° C. many substances produce such marked increase of tone, that the response to all forms of stimulation is diminished, hence to prevent this increase of tone, these experiments were performed at 18–20° C.

Mammalian saline (Singh, 1940) was used. A difficulty in these experiments is that a long time, 3 to 6 hours, might be required to get a constant response. This time was least at pH 8, using borate buffer. Hence at this pH, borate buffer was used. Response is maximum at pH 8 and at pH 6·5. Hence to test the effect of excess of calcium and hydrogen-ions, the saline was buffered with phosphate at pH 6·5. To increase the concentration of hydrogen-ions further, salines buffered with phosphate at pH 6, 5·5 and 5·2 were used. The experiments with excess of calcium were also performed at pH 8. All other experiments were performed at pH 8.

Results

Effect of anions

Effect of bromide.—Replacement of the chloride of the saline with bromide produces a tonic contraction, which however is slight at 18 to 20° C., the temperature at which these experiments were performed, and hence it does not interfere with the responses of the muscle to electric current, nervous stimulation, acetylcholine or potassium. Such replacement decreases the response to alternating current (Fig. 1), and increases that to acetylcholine

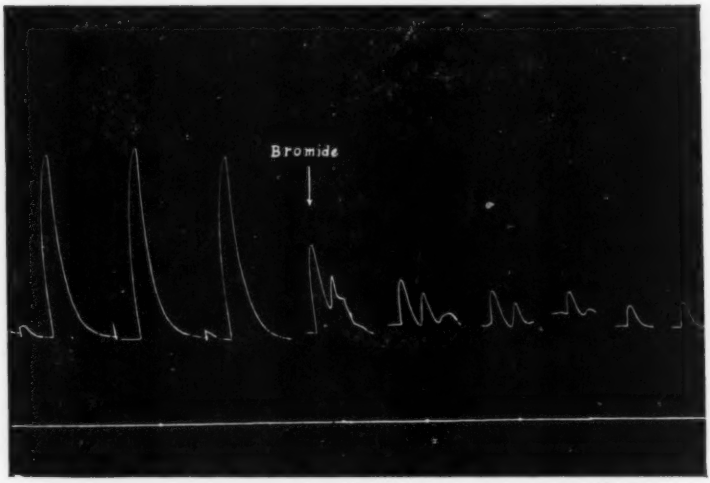

Fig. 1. Dog's stomach muscle. Effect of replacement of the chloride of the saline with bromide on the response to alternating current (12 volts/10 sec.). Response every 15 min.

(Fig. 2) and to potassium which produces responses similar to those produced by acetylcholine (Fig. 3). The response to nervous stimulation is decreased (Fig. 4). Small concentrations of bromide, about 5 per cent,

replacement of the chloride, may increase the response to alternating current. Complete replacement of the chloride of the saline with bromide shows that the response to nervous stimulation resembles that produced by electric current and not those produced by acetylcholine or potassium.

Effect of nitrate.—Replacement of the chloride of the saline with nitrate causes tonic contraction. Nitrate increases the response to acetylcholine and potassium and decreases the response to nervous stimulation (Fig. 4).

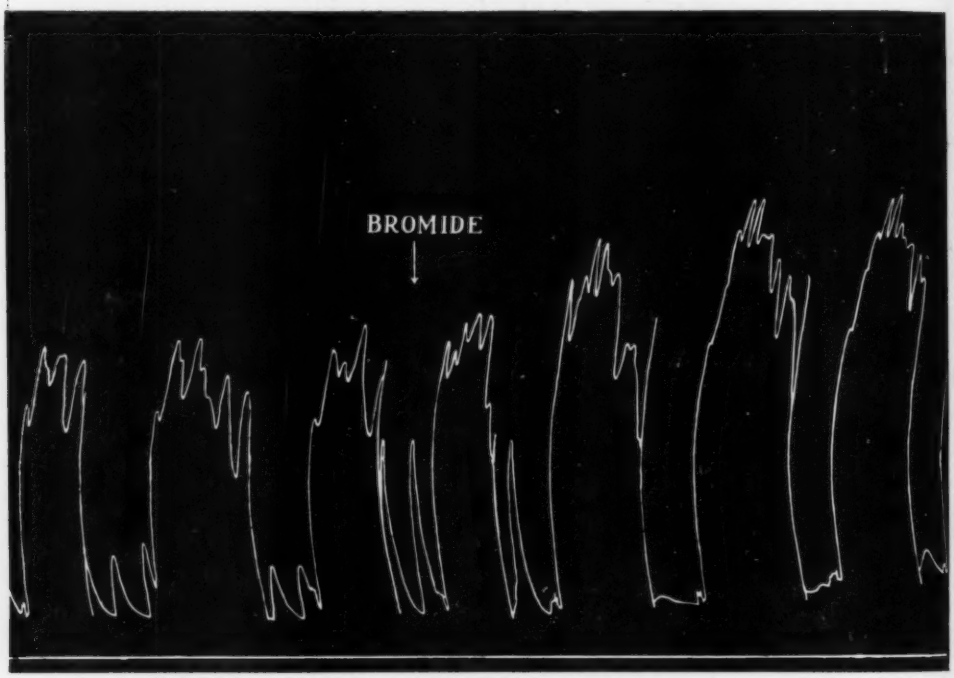

FIG. 2. Dog's stomach muscle. Effect of replacement of the chloride of the saline with bromide on the response to acetylcholine (1 in 10^8). Response every 15 min.

The response to electric current is variable, it may increase or decrease, or increase at first and then decrease. The response to nervous stimulation thus resembles more that to electric current than to acetylcholine or potassium.

Effect of iodide.—Replacement of the chloride of the saline with iodide causes tonic contraction. Iodide increases the response to acetylcholine (Fig. 5), potassium (Fig. 6), and decreases that to nervous stimulation (Fig. 4).

The response to electric current decreases from the outset or it may first increase and then decrease (Fig. 7). The response to nervous stimulation therefore resembles that produced by electric current.

Effect of thiocyanate.—Replacement of the chloride of the saline with thiocyanate produces tonic contraction like other anions. Thiocyanate

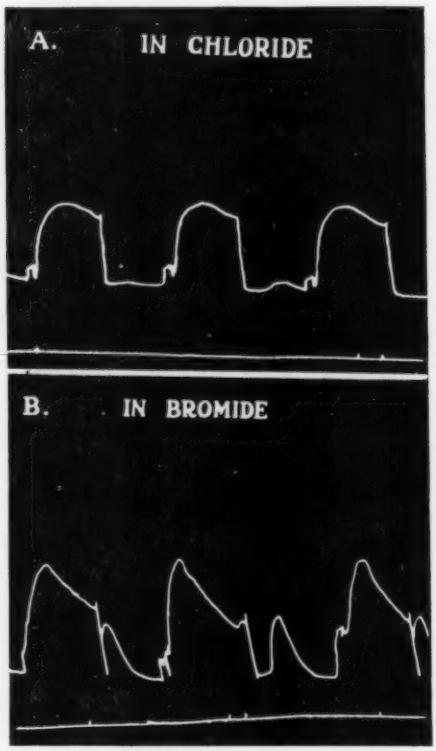

FIG. 3. Dog's stomach muscle. Effect of replacement of the chloride of the saline with bromide on the response to potassium. Response every 15 min. A. Responses in chloride. B. Responses in bromide.

decreases the response to nervous stimulation (Fig. 4). The response to electric current is at first increased and then decreased (Fig. 8). The response to potassium and acetylcholine is increased (Fig. 9). The response to nervous stimulation thus resembles that to electric current and not that produced by acetylcholine or potassium.

Effect of cations

Effect of calcium.—In dog's stomach muscle, excess of calcium ions produce tonic contraction, which may be marked in alkaline solutions at pH 8; this is greatly reduced if the temperature is kept at 18–20° C. The

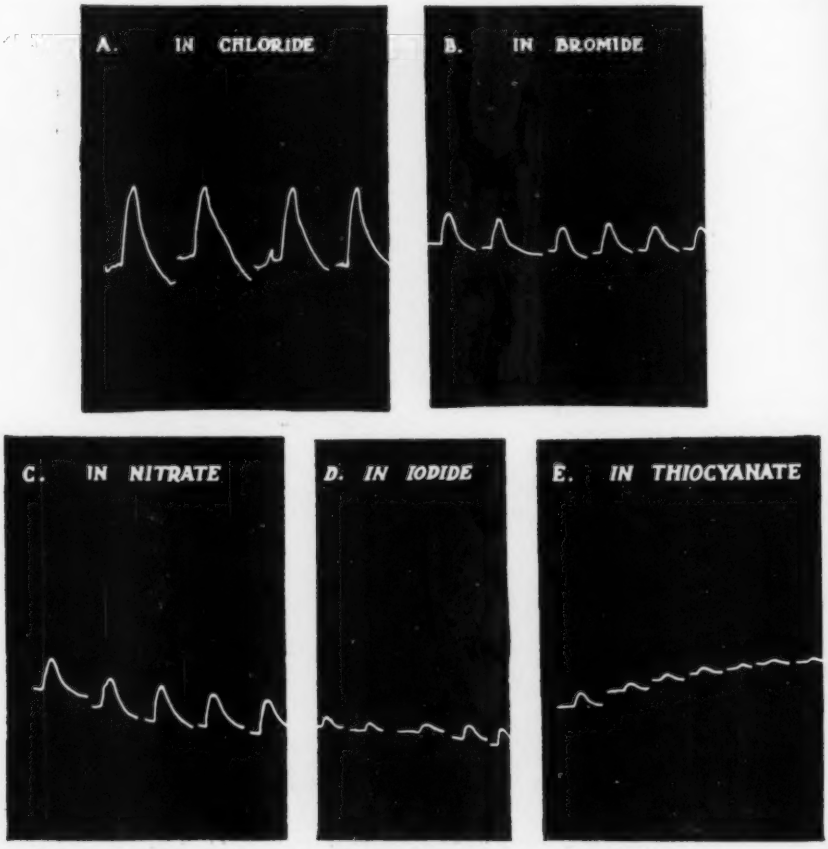

FIG. 4. Dog's stomach muscle. Effect of replacement of the chloride of the saline with other anions on the responses to nervous stimulation for 15 sec. every 15 min. A. Responses in chloride. B. Responses in bromide. C. Responses in nitrate. D. Responses in iodide. E. Responses in thiocyanate.

effect of calcium ions was tested both in alkaline as well as acid solutions (pH 7–6·5).

In acid solutions, excess of calcium ions potentiate the response to potassium, but antagonise that to electric current (Singh, 1940). The optimum

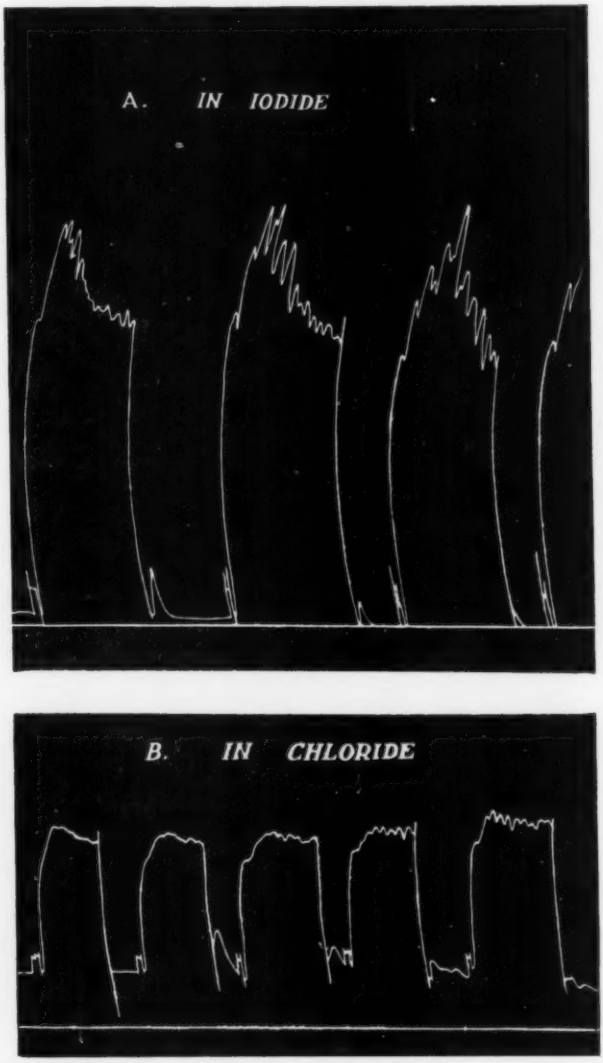

FIG. 5. Dog's stomach muscle. Effect of replacement of the chloride of the saline with iodide on the response to acetylcholine (1 in 10^5). Response every 15 min. A. Responses in iodide. B. Responses in chloride.

concentration of calcium for the response to electric current in acid solutions is 0·004 *M*; beyond this the response decreases (Fig. 10). The response

to acetylcholine increases in excess of calcium. It gives a marked response in pure solutions of calcium chloride (Fig. 11). As the concentration of calcium is increased beyond 0·004 *M*, the response to acetylcholine may

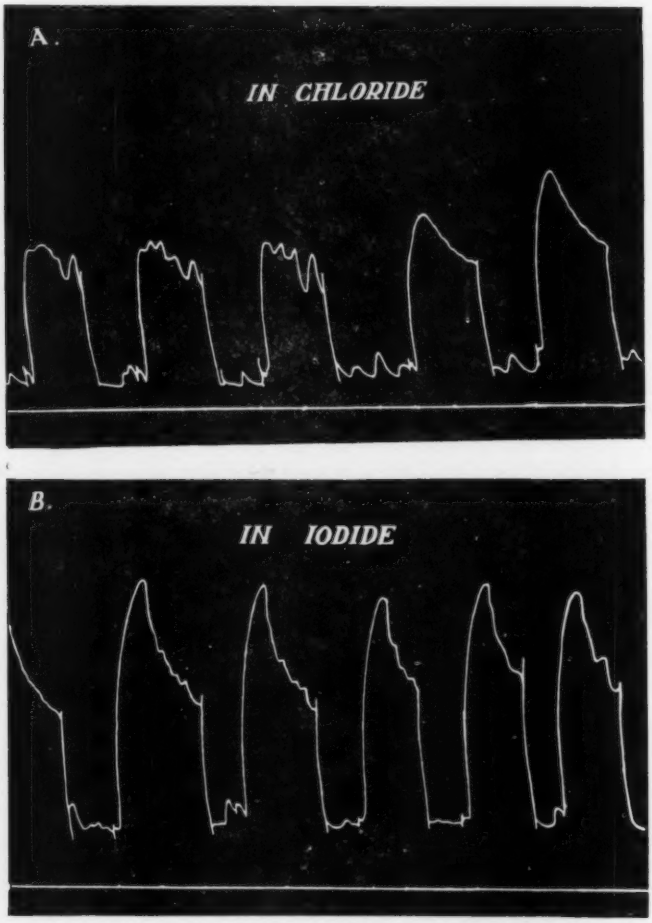

FIG. 6. Dog's stomach muscle. Effect of replacement of the chloride of the saline with iodide on the response to potassium. Response every 15. min. A. Responses in chloride and iodide. B. Responses in iodide.

slightly increase after a preliminary increase, and then increase again. This slight decrease is presumably due to increased accommodation produced by calcium.

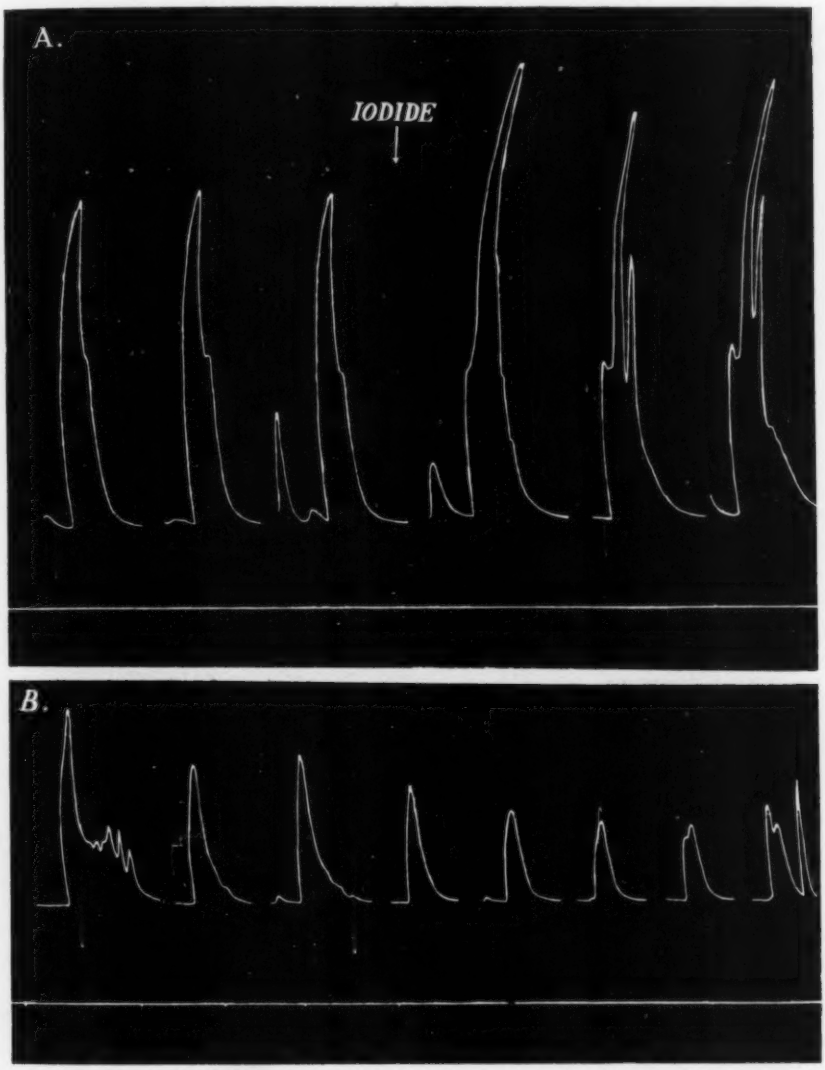

FIG. 7. Dog's stomach muscle. Effect of replacement of the chloride of the saline with iodide on the response to alternating current (12 volts/10 sec.). Response every 15 min. A. Responses in chloride and iodide. B. Responses in iodide.

In acid solutions the response to nervous stimulation increases up to 0·008–0·01 *M*, and then decreases, but may be obtained in excess of calcium ions up to 0·06 *M* (Fig. 12). There is no doubt, therefore, that excess of calcium ions potentiate the response to acetylcholine, potassium and nervous stimulation, but decrease that to electric current. The final suppression of the response to nervous stimulation in excess of calcium is presumably due to interference with neuromuscular transmission.

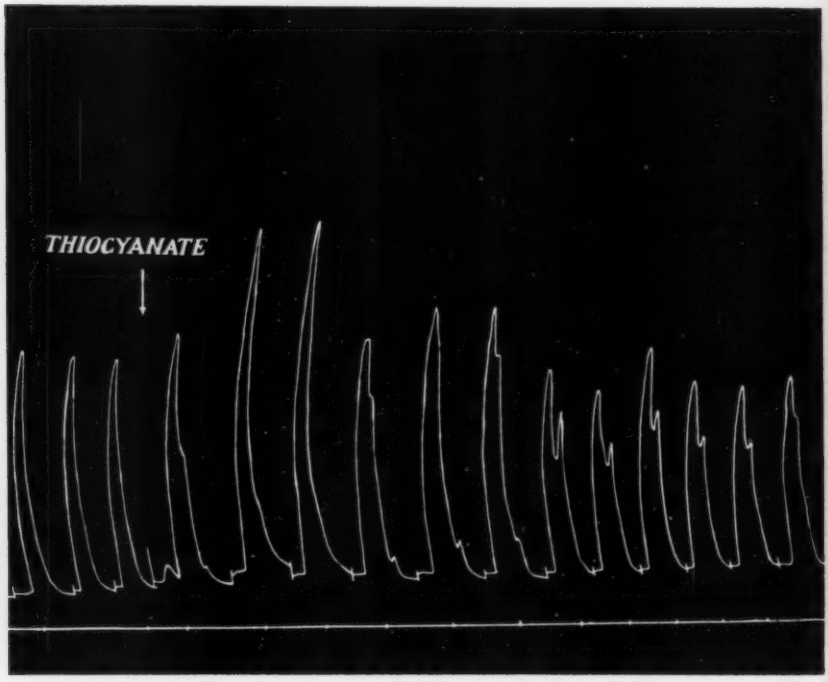

FIG. 8. Dog's stomach muscle. Effect of replacement of the chloride of the saline with thiocyanate on the response to alternating current (12 volts/10 sec.). Response every 15 min.

In alkaline solutions, pH 8, excess of calcium produces a tonic contraction, which however decreases as the concentration of calcium is increased. Calcium is antagonistic to tonic contraction, hence when in excess, decreases its own response. The response to alternating current sharply decreases, if the concentration of calcium exceeds 0·002 *M* (Fig. 13). The response to acetylcholine is increased in excess of calcium, up to a certain value (Fig. 14). The response to nervous stimulation also increases in

excess of calcium, though it may decline at first owing to increase of tone (Fig. 15).

Effect of hydrogen-ions.—If the concentration of hydrogen-ions is increased beyond 6·5, the response to electric current, acetylcholine and nervous stimulation decreases, hence hydrogen-ions are not useful in differentiating these responses.

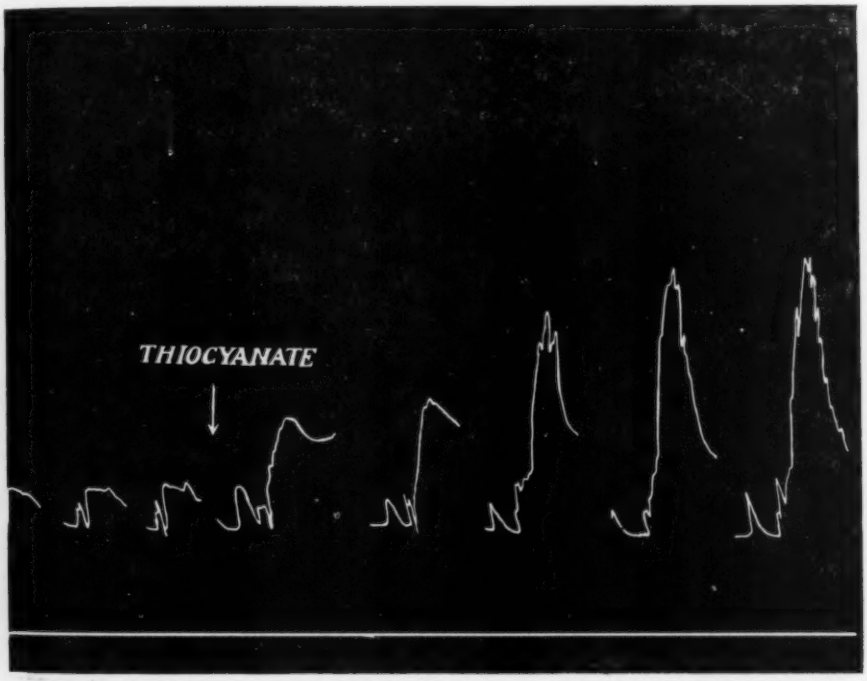

FIG. 9. Dog's stomach muscle. Effect of replacement of the chloride of the saline with thiocyanate on the response to acetylcholine (1 in 10^8). Response every 15 min.

DISCUSSION

Neuromuscular transmission is in its simplest form in unstriated muscle as there are no intervening motor end plates as in skeletal muscle, or complicated synapses as in the central nervous system. The nerve fibres appear to end directly in relation to the muscle fibres.

The anions produce a tonic contraction, hence they antagonise the response to electric current (Singh, 1938 *a*), but potentiate the response to other

tone-producing substances, such as acetylcholine and potassium. They antagonise the response to nervous stimulation, which shows that it is produced by electric current, and this must be the action potential of nerve.

The effect of excess calcium shows that nervous stimulation results in liberation of some tone-producing substance, which is presumably acetylcholine. The initial action potential therefore produces a phasic response,

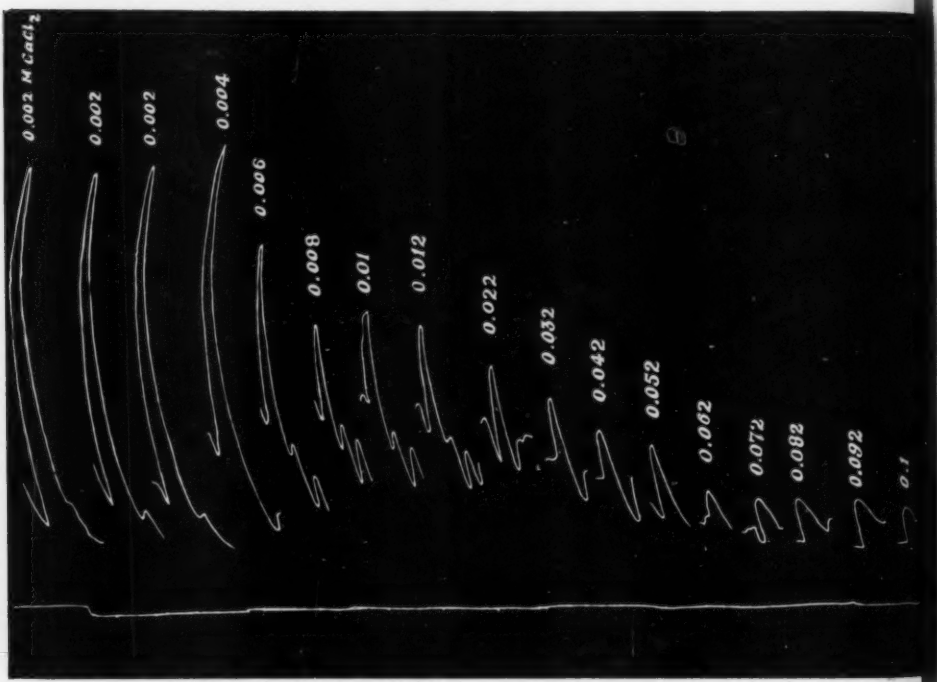

FIG. 10. Dog's stomach muscle. Effect of excess of calcium on the response to alternating current (12 volts/10 sec.). Response every 15 min. pH 6·6, phosphate.

which however is sustained by the subsequent liberation of acetylcholine. This process resembles the sympatheticoadrenal response, the sympathetic producing its effects by nervous stimulation, which are sustained by the hormones from the adrenal medulla.

These experiments show that there are two components in transmission, one electrical and the other chemical, one of which may be suppressed. Thus transmission in various parts of the body may be electrical, chemical or electro-chemical. If nervous stimulation is abolished by atropine, then the

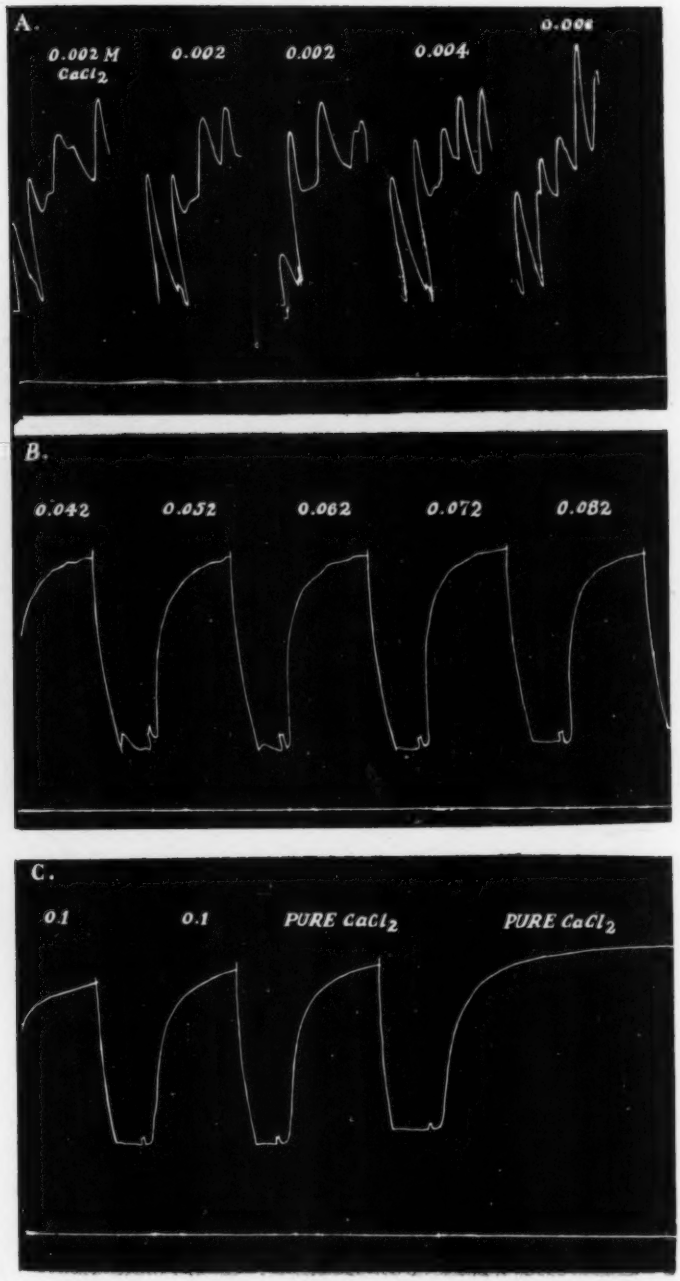

FIG. 11. Dog's stomach muscle. Effect of excess calcium on the response to acetylcholine (1 in 10^8). Response every 15 min. pH 6·6, phosphate.

transmission is presumaly electrical. If it is not abolished by atropine, then it presumably electro-chemical, as no substance has been found which suppresses the response to electric current so completely as atropine suppresses that produced by acetylcholine in dog's stomach muscle, leaving other excitabilities unaffected.

That the response to nervous stimulation is not purely due to the liberation of acetylcholine is shown by the fact that the responses to nervous and

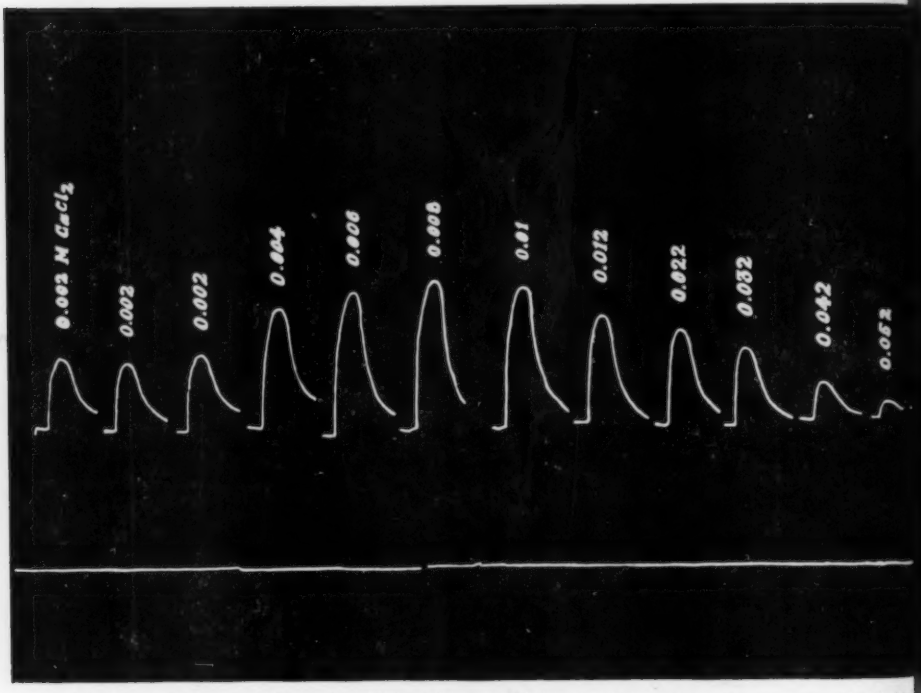

FIG. 12. Dog's stomach muscle. Effect of excess calcium on the response to nervous stimulation for 15 sec. every 15 min. pH 6·6, phosphate.

chemical stimulation may be affected oppositely. Thus in frog's stomach muscle, small concentrations of atropine may potentiate the response to electric current and nervous stimulation, but completely suppress the response to acetylcholine (Singh and Singh, 1947). In frog's stomach muscle, small concentrations of eserine sulphate have a peculiar effect, that the response to acetylcholine may be completely suppressed, but that to electric

current and nervous stimulation potentiated. Adrenaline also has similar action.

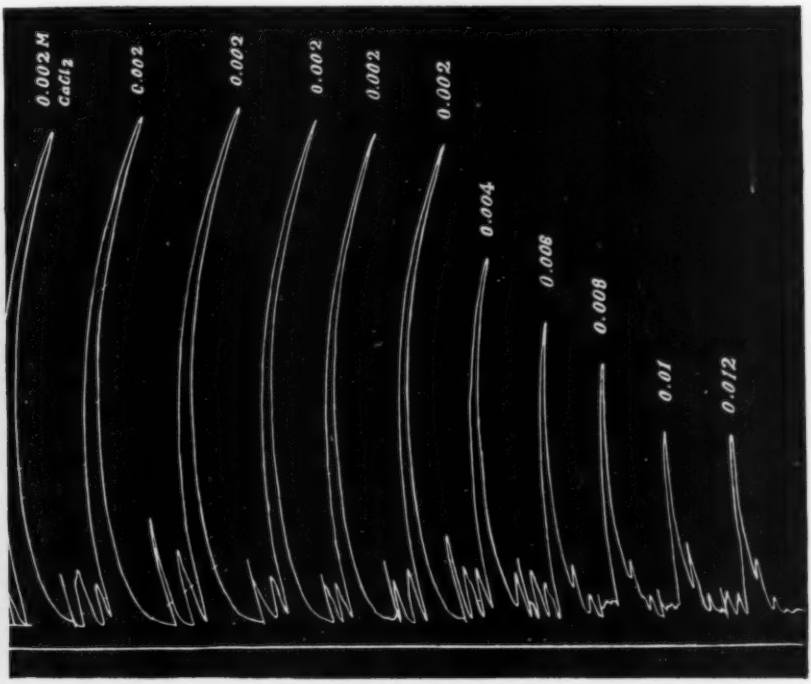

FIG. 13. Dog's stomach muscle. Effect of excess calcium on the response to alternating current (12 volts/10 sec.). Response every 15 min. pH 8, borate.

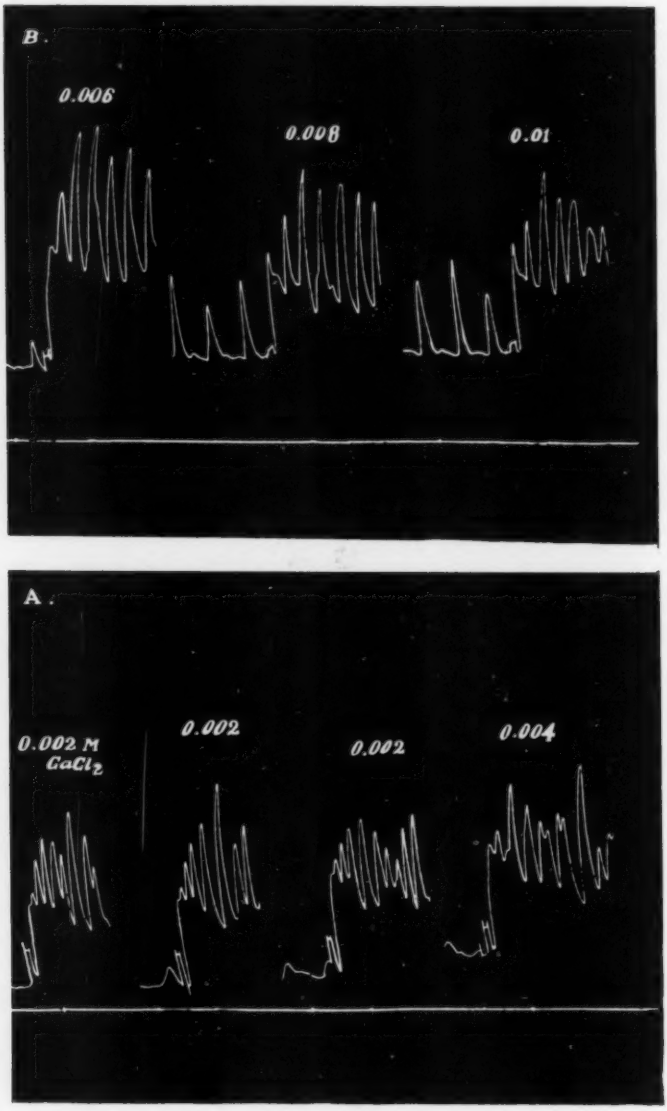

FIG. 14. Dog's stomach muscle. Effect of excess calcium on the response to acetylcholine (1 in 10^8). Response every 15 min. pH 8, borate.

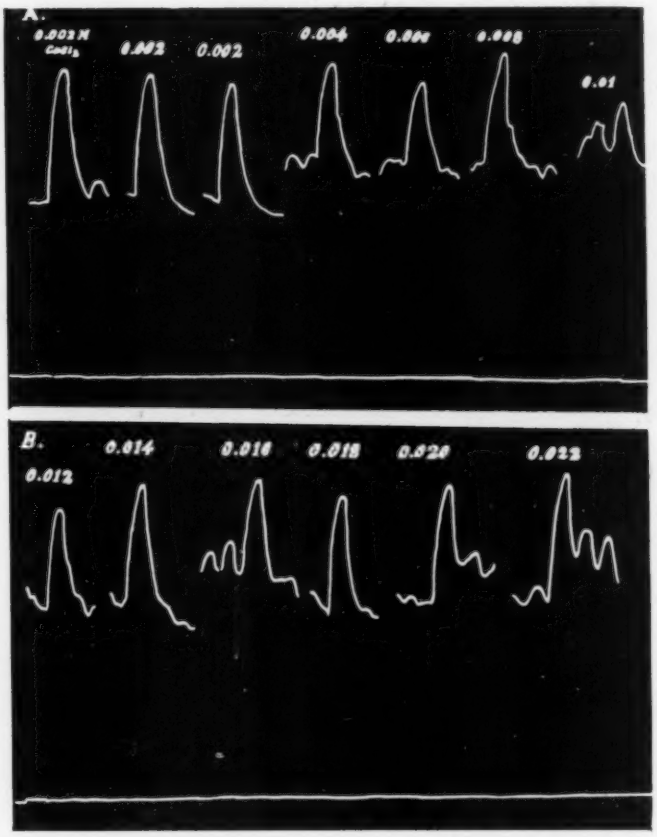

FIG. 15. Dog's stomach muscle. Effect of excess calcium on the response to nervous stimulation for 15 sec. every 15 min. pH 8, borate.

SUMMARY AND CONCLUSIONS

1. Unstriated muscle shows two kinds of responses; one kind is produced by electric current and the other by chemicals such as potassium or acetylcholine.

2. Certain reagents affect these two kind of responses differentially.

3. In dog's stomach muscle, the responses produced by electric current are inhibited by tone-producing substances such as bromide, nitrate, iodide, thiocyanate. The responses to chemical stimulation are potentiated. The

response to nervous stimulation are inhibited. It is concluded, therefore, the response produced by nervous stimulation is produced by electric current, and that can only be the action potential.

4. Excess of calcium potentiates the response to nervous stimulation, acetylcholine and potassium, but inhibits that to electric current. So it is concluded that the response due to nervous stimulation is also due to liberation of a chemical substance, presumably acetylcholine.

5. Neuromuscular transmission in unstriated muscle is therefore electro-chemical, produced both by the action potential of nerve and liberation of a chemical substance. The former produces the initial response, and the latter sustains it, and imparts to it, tonic properties.

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A CHROMOSOME SURVEY OF INDIAN DIOSCOREAS

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Before the advent of the potato into Asia Dioscorea, popularly known as Yams, occupied an important part in the dietics of the people of the Tropics and many cultivated forms have arisen from the wild species which are found growing in the forests. These form the staple food of some of the primitive tribes of India who still collect them with "digging sticks". Dioscorea is also important medicinally because of the presence of glucosides, saponins and the alkaloid dioscorine (Chopra *et al.*, 1956). Recently the tubers of some have been found to be the starting point for the manufacture of cortisone.

At the Central Botanical Laboratory a cyto-geographical survey of the species of Dioscorea found in India is being made, to trace the chromosome relationship between the wild and the cultivated forms and to see the evolutionary sequence following selection by primitive man in the conversion of a wild to a cultivated plant. South India offers a very fertile field for this study as we have several tribes in Kerala and Madras who are still in the neolithic age and gather food with "digging sticks". Such are the Kadars of the Annamalais, and the Pannyars of Wynaad, while the more civilized tribes like the Erulas of Coimbatore supplant their diet of grain with the Yam on which they subsisted at an earlier stage.

More than 100 tubers covering nine species have been collected from the following regions—Annamalai hills, Palghat, Shoranur, Tellicherry and from the hills surrounding Coimbatore as well as from Bombay, Calcutta, Dehra Dun, Mussouri, Poona and Lucknow.

The tubers were grown in pots and root tips were fixed in chrome-acetic formalin, sectioned at 12μ and stained in crystal violet. The present paper is only a preliminary report of the work done at the Central Botanical Laboratory and a detailed description of the work done will be published elsewhere.

TABLE I

Chromosome numbers in the genus Dioscorea

Sl. No.	Name	Vernacular name	Collected from	Chromosome Nos.	Previous observations	Remarks
1	*D. deltoidea* Wall.	Kirta	Dehra Dun	$2n=40$		Twines to the left, wild. Tubers contain saponin, used as fish poison and to kill lice.
2	*D. hispida* Dennst. syn. *D. Dæmona* Roxb. *D. triphylla* L.	Peiperandai Erumaikilangu	Annamalais Annamalais Shoranur Calcutta	$2n=40$ $2n=40$ $2n=40$ $2n=40$		Twines to the left, wild. Contains poisonous alkaloid dioscorine (Prain and Burkill, 1911). Can be cooked after repeated washing for 3 days in running water so as to remove all traces of the alkaloid. Bulbils never seen. Used as arrow poison.
3	*D. pubera* Bl.	Aduthappakachil	Vadakara, Travancore	$2n=40$		Wild Twines to the right. Tubers small, spherical, edible. Bulbils form freely.
4	*D. oppositifolia* L.	Vetrilaivalli ..	Coimbatore Annamalais	$2n=40$ $2n=40$	$2n=ca.$ 140 (Smith, 1937)	Twines to the right, wild. Tubers long, lie very deeply buried. Never forms bulbils. Edible and favoured by the Erulas. Said to be a cure for scorpion bite.
5	*D. pentaphylla* L. var. *linnæi* Prain and Burkill *D. pentaphylla* L. var. *jacquemontii* Prain and Burkill. *D. pentaphylla* L.	Mulluvalli .. Noorankilangu Mulluvalli .. Sorian Noorankilangu ..	Coimbatore Bombay Travancore Coimbatore Shoranur Annamalais Shoranur Sibpur, Calcutta	$2n=40$ $2n=80$ $2n=80$ $2n=40$ $2n=80$ $2n=80$ $2n=80$ $2n=80$	$2n=ca.$ 144 (Smith, 1937)	Twine to the left, bulbils freely formed, wild but edible. Tubers lie deeply buried. In var. *linnæi* tubers are as long as 1 metre in length. Used medicinally in the treatment of swellings.

6	*D. alata* L. ..	Rathalu	Lucknow	$2n=40$	$2n=81$ (Nakajima, 1936)	Twine to the right. Bulbils freely formed, commonly cultivated. Among the cultivated forms, two distinct types could be recognized, the white variety and the magenta type. The magenta type is again of two kinds, in one the red water-soluble pigment is restricted to the periphery alone, while in the other it is diffused throughout the cells. Both the white and the magenta type have the same polyploid series. The completely red type of tuber known as Chuoanakachil has $2n=40$ chromosomes. Used medicinally in the case of ulcers and dysentery.
		Rathalu	Sultanpur	$2n=40$	$2n=40$ (Simmonds 1954)	
		Perumvallikilangu	Coimbatore	$2n=40$	$2n=30, 40, 50, 70$	
		Chuoanakachil	Vadakara, Kerala	$2n=40$	(Sharma and De, 1956)	
		Parichkodan	do.	$2n=40$		
		Maravattikilangu	Kuttattukulam, Kerala	$2n=40$		
		Mattukachil	Kerala	$2n=40$		
		Injikachil	Vadakara, Kerala	$2n=40$		
		Kavathu	Shoranur	$2n=40$		
		Rathalu	Lucknow	$2n=60$		
		Muchikavathu	Travancore	$2n=60$		
		..	Sultanpur	$2n=80$		
		..	Dehra Dun	$2n=80$		
		..	Calcutta	$2n=80$		
		..	Simla	$2n=80$		
		Perumvallikilangu	Coimbatore	$2n=80$		
		..	Shoranur	$2n=80$		
7	*D. bulbifera* L. syn. *D. sativa* Thunb. *D. pulchella* Roxb.	Pannikilangu	Annamalais	$2n=40$	$2n=80$ (Nakajima 1936)	Twine to the left. Bulbils freely formed, wild. Tubers not so much elongated but more or less spherical. Contains poisonous glucosides. Medicinally useful in cases of ulcers and piles. Edible.
		do.	Annamalais	$2n=80$		
		Kodikilangu	Coimbatore	$2n=80$		
		Kattukilangu	Coimbatore	$2n=80$		
		..	Powai Lake, Bombay	$2n=80$		
		..	Poona	$2n=80$		
		..	Mussouri	$2n=80$		
		..	Dehra Dun	$2n=80$		
		..	Calcutta	$2n=80$		
		..	Shoranur	$2n=80$		
		..	Shoranur	$2n=ca.$ 98–100		
8	*D. belophylla* Voigt	Torar, Tarri	Calcutta	$2n=80$	..	Twine to the right. Bulbils abundantly produced. Tubers approximately 20 cm. in length, lie deeply buried in the soil and edible.
9	*D. esculenta* Burkill. sin. *D. aculeata* L. *D. fasciculata* Roxb.	Nanakilangu	Palakuzha, Kerala	$2n=90$		Twine to the left. Bulbils never form. Only cultivated varieties. Tubers small round 2″ to 6″ long and ¾″ to 1¼″ broad. Tubers formed very near the surface of the soil. Used in the treatment of swellings.
		Rathalu	Sultanpur	$2n=90$		
		do.	Dehra Dun	$2n=90$		
		Musilamvallikilangu	Annamalais	$2n=90$		
		do.	Annamalais	$2n=90$		
		Siruvallikilangu	Coimbatore	$2n=ca.$ 100		
		Cherukilangu	Vadakara, Kerala	$2n=100$		
		Rathalu	Lucknow	$2n=ca.$ 100		

A list of the chromosome counts of the species examined is given in the above table.

It will be seen from the list that the South Indian races of *D. oppositifolia* and *D. pentaphylla* seem to be lower polyploids than those examined by Smith (1937).

In the case of *D. bulbifera*, the Annamalai form has the lowest chromosome number while the plant collected from Shoranur has as high as 100 chromosomes.

The chromosome number of the cultivated *D. esculenta* is much higher than the wild forms studied so far.

SUMMARY

The present paper deals with the chromosome numbers of nine species of wild and cultivated Yams. Five species, namely, *D. pubera*, *D. deltoidea*, *D. hispida*, *D. belophylla* and *D. esculenta*, have been worked out for the first time. In *D. alata* and *D. bulbifera* different chromosome races for the Indian forms have been recorded besides the ones reported by previous workers. It is interesting to observe that the Japanese races of *D. oppositifolia* and *D. pentaphylla* are higher polyploids than the Indian forms reported here.

ACKNOWLEDGEMENT

I wish to record my thanks to Dr. S. K. Mukherjee, Keeper, Central National Herbarium of the Botanical Survey of India, who has been so good as to identify and verify the herbarium sheets of all plants whose chromosome numbers have been reported in this paper.

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EXPLANATION OF PLATES

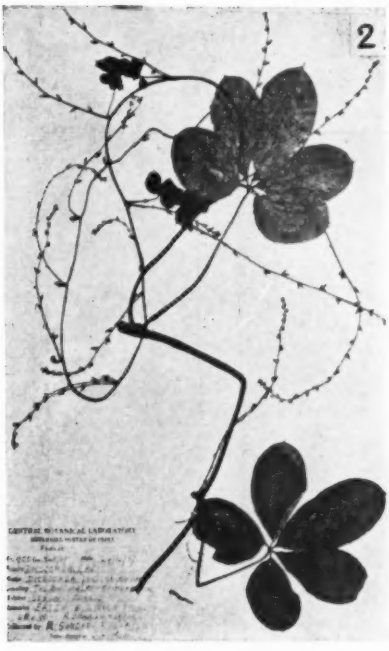
PLATE V. Photographs of Herbarium Sheets.

PHOTO 1. *D. pentaphylla* var. *linnæi*, Prain & Burkill (Coimbatore, $2n = 40$).

PHOTO 2. *D. pentaphylla* Linn. (Coimbatore, $2n = 80$).

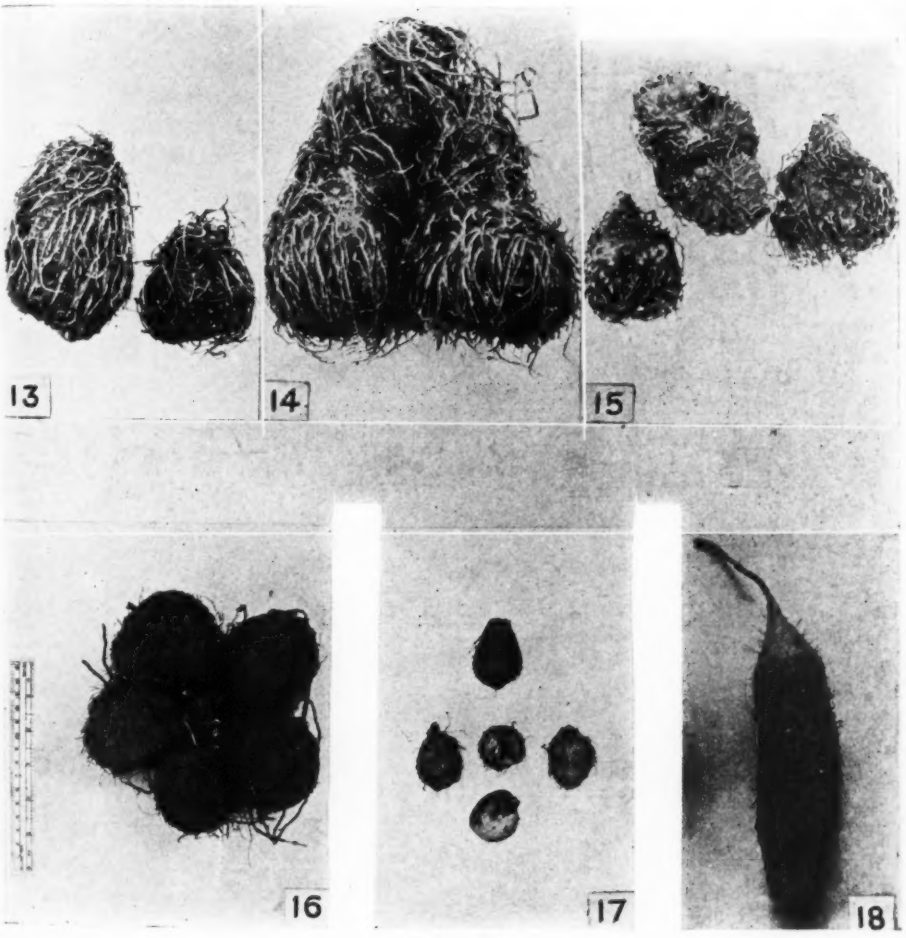
PHOTO 3. *D. oppositifolia* L. (Annamalais, $2n = 40$).

PHOTO 4. *D. bulbifera* L. (Bombay, $2n = 80$).



FIGS. 1–4

FIGS. 5–12



FIGS. 13–18

PLATE VI. Photographs of Living Tubers of Dioscorea.

PHOTO 5. *D. oppositifolia* L. (Coimbatore—Vetrilaivalli, $2n = 40$).

PHOTO 6. *D. pentaphylla* var. *jacquemontii*, Prain & Burkill (Coimbatore, $2n = 40$).

PHOTO 7. *D. pentaphylla* var. *jacquemontii*, Prain & Burkill (Shoranur, Kerala, $2n = 80$).

PHOTO 8. *D. pentaphylla* Linn. (Shoranur—Noorankilangu, $2n = 80$).

PHOTO 9. *D. alata* Linn. (Kerala, Chuoanakachil, $2n = 40$). Tubers completely red like Beta *vulgaris*.

PHOTO 10. *D. alata* Linn. (Lucknow, Rathalu, $2n=40$). Tubers white, cultivated and very long.

PHOTO 11. *D. alata* Linn. (Kerala, $2n = 60$). Tubers have magenta colour limited to the periphery alone.

PHOTO 12. *D. alata* Linn. (Coimbatore, $2n = 80$).

PLATE VII. Photographs of Living Tubers of Dioscorea.

PHOTO 13. *D. bulbifera* L. (Annamalais, Parnikilangu, $2n = 40$).

PHOTO 14. *D. bulbifera* L. (Bombay, $2n = 80$).

PHOTO 15. *D. bulbifera* L. (Shoranur, Kerala, $2n = ca.$ 100).

PHOTO 16. *D. hispida Dennst.* (Annamalais, Peiperendai, $2n = 40$).

PHOTO 17. *D. esculenta* Burkill. (Kerala, Nanakilangu, $2n = 90$).

PHOTO 18. *D. esculenta* Burkill. (Lucknow, Rathalu, $2n = ca.$ 100). Tubers longer than other cultivated varieties and very spiny.

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STUDIES ON THE ANATOMY OF THE SUGARCANE MOTH BORERS

V. Comparative Anatomy of the Adult Thorax

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1. Introduction

Although the anatomy of the different sugarcane moth borers of India has been a subject of study for quite a few workers in the past, no serious attempt appears to have been made to elucidate on a comparative basis the complete anatomy of these insects in detail. In particular, the thorax of these insects has received very little attention and no absolute descriptions of the same are available. Gupta (1940) studied and described the anatomy of four different species of striped moth borers of sugarcane found in North

Bihar and Western Uttar Pradesh, *viz.*, *Chilotræa infuscatellus*, *C. auricilia*, *Proceras indicus* Kapur and *Chilo zonellus* but his descriptions pertaining to the thorax of these insects are too brief. He has recorded only three species out of seven recorded in the State of Bihar as serious pests of sugarcane. Kapur (1950) dealt with the revision of taxonomy of different crambinid moths of sugarcane but did not describe the detailed anatomy of their thorax. Imms (1951) is of the opinion that the imagines of Lepidoptera exhibit a remarkable constancy as regards their fundamental structure in the order. This appears to be true of the insects under study but for the few useful and interesting differences being presented below which have not been noticed and described before. Cheema (1951) reproduced a very brief description of the adult moth of *Emmalocera depressella* with little differences of colour, etc.; already described by Hampson (1896). Krishnamurti and Usman (1952) gave a brief description of the anatomy of thorax in case of *Sesamia inferens* without any illustration of the parts thereof. Puri (1957) worked out in detail the external anatomy of *Chilo tumidicostalis*, a stem borer of sugarcane in Bihar.

The object of the present contribution is to discuss the comparative anatomy of the thorax of eight different species of sugarcane moth borers, *viz.*, *Scirpophaga nivella*, *Chilo tumidicostalis*, *C. zonellus*, *Chilotræa infuscatellus*, *C. auricilia*, *Bissetia steniellus*, *Sesamia inferens* and *Emmalocera depressella* and to provide a basis for further studies needed to bridge existing gaps in the knowledge of anatomy of thorax in these insects. Important in this connection may be mentioned the study of characters that might be helpful in the identification of different species as also in tracing out their phylogenetic relationships with one another.

2. MATERIAL AND METHODS

The moths of seven different species, *viz.*, *Scirpophaga nivella*, *Chilo tumidicostalis*, *C. zonellus*, *Chilotræa infuscatellus*, *C. auricilia*, *Sesamia inferens* and *Emmalocera depressella* were reared in the laboratory at Pusa. Those of *Bissetia steniellus* were supplied by the Cane Commissioner and the Entomologist to Punjab Government and also by the Sugarcane Entomologist, Shahjahanpur.

The study of the thorax was carried on after soaking the specimens in 10% KOH solution, for about 24 hours, washing under a current of water for about 8 hours, dehydrating, staining with eosin in 90% alcohol, clearing in carbo-turpentine, turpentine, cedar wood oil and finally mounting them in canada balsam as suggested by Puri (1954). It has been observed that the complete internal tissues could not be squeezed out completely in order

to make the specimens clear and transparent in case of *Bissetia steniellus* as the specimens were old and completely dried up. The photographs reproduced in the present paper are direct microphotographs from the permanent slides of various structures.

3. Description of Different Parts

(i) *Cervix*

The intersegmental region which is almost entirely membranous forms the neck or the cervix. The two sclerotized lateral cervical sclerites, in the form of V (laterally placed), are quite prominent. Each lateral cervical sclerite (Plate VIII, Fig. 1–9) appears to be composed of two pieces or arms arranged in such a way so as to form a V-shaped lateral cervical sclerite. The two arms of each sclerite are hinged together externally at a point that forms the apex of the letter V which articulates with the anterior margin of the prothoracic episternum and is prolonged beyond the point of articulation as a short thick free stem. The distal ends of the anterior or dorsal ends of each sclerite are produced into peg-like projections to fit into the concavities of the occipital condyles of their respective sides, thus affording an articulation between the head and the neck at the points on which the former can move freely. The posterior or the ventral arms of these sclerites articulate with the prothoracic episternum laterally and appear to be united by a narrow bridge.

(ii) *Thorax*

The general structure of the thorax of all the eight species of these moths is almost of similar type but for the minor differences in the shape and form of the convergent ridges in the metathorax, the presence or the absence of the median carina in the mesoscutum. These are the only characters which can be said to be useful in the proper identification of these species so far the thorax is concerned. The convergent ridges are comparatively well developed in the *Pyralidæ* than in the *Noctuidæ*. This is probably due to the fact that the formers are swift fliers. The observation that the length of the convergent sutures is more in the swift fliers in the order Lepidoptera needs further investigations before it can be established as a fact.

(*a*) *Prothorax*.—The prothorax (Plate VIII, Figs. 4 and 5; Plate IX, Fig. 11) may be composed of three pronotal plates which are arranged in such a way that mesally these give rise to a Y-shaped structure in between the two lateral lobes formed by these plates and their lateral outgrowths or the patagia. The free stem or the posterior elongation of the letter Y is produced into a vertical peg-like projection articulating posteriorly with

a concavity on the mesothoracic pre-scutum and appears to be partially fused with the episternal sclerites.

The pronotal plates are comparatively much reduced and fused together in *Sesamia inferens*. The patagia in all these species are somewhat similar structures but for little differences in their length and breadth in these species. There are no larger lobes referred to as parapatagia by Madden (1944) behind the patagia in these insects.

The ventral margins of the pronotum are fused with the episternum which is somewhat a convex sclerite. A distinct pleural suture is quite obvious and forms a prominent pleural ridge which in turn gives rise to a long slender pleural arm. The two pleural arms of both the sides join the sternal furca near the sternal pits (Plate VIII, Fig. 3). The anterior portion of the sternum is fused with the episternum by a faint and narrow pre-coxale and the post-coxales attain their separate entity and connect the epimeron with the sternum, behind the coxal cavity on both the sides.

The presternum is also well developed and runs horizontally as an internal apodeme. It is partially fused with the episternum and also connected with the basisternum and sternellum by the so-called strap-like spinasternum. At its sides it also receives the post-coxale and the inturned ventral edge of the trochantin.

The edges of a deep median fold of the transverse internal ridge or the sternacosta represent the reduced basisternum. It is separated from the sternellum by a well-marked sternacostal suture which extends right up to the sternal pits in the form of a broad U.

The spinasternum is represented by a vertical median strap-like process which forks into two lateral arms posteriorly which almost touch the anterior margin of the mesothoracic basisternum.

The trochantin is a large plate bounded by the pleural arms, its own inturned ventral edge and the post-coxale.

(*b*) *Mesothorax*.—The largest and the most prominent of the three segments of the thorax in these insects is the mesothorax (Plate IX, Figs. 1–11). The pre-scutum lies anterior to the scutum, directed ventrad and is almost invisible from the dorsal aspect. A narrow bowl-shaped cavity occurs near its anterior margin almost in the centre. Laterally its margins are produced into two lateral prealares which form the prealare bridge. The pre-scutal ridge formed by the pre-scutal suture sets off the pre-scutum from the scutum.

The scutum (Plate IX, Figs. 1–11) as usual is very prominent, being the largest of the thoracic tergites in all these species. Its anterior margin is cleft and the lateral margins caudad to the prealares, providing for the incision of the notal sub-tegular wing processes or arms. A little beyond this point, each lateral margin is produced or flattened into a suralare which serves as an anterior pivotal point for the wings.

The median suture which forms the median carina is well developed in *Scirpophaga nivella*, *Chilotræa infuscatellus* and *Emmalocera depressella* while in other species it is either very feebly developed or completely obliterated as is the case in *Sesamia inferens* (Plate IX, Fig. 10) and *Chilo tumidicostalis* (Plate IX, Fig. 2).

The notal sub-tegular wing processes are well developed in all these species and have been described in detail by Puri (1958).

The two lobe-like outgrowths are the postero-lateral extensions of the scutum on either side. The anterior adanale is long and slender and serves as the posterior articulatory point for the wings while the post-adanale is represented by the shorter and stouter posterior lobe. The lateral margin of the scutum between the suralare and the adanale is extended and projected forward into an adnotale which also functions as an articulatory point for the wing. The axillary cords of the basal wing membranes seem to arise at a point just posterior to the post-adanales, at the sides of the posterior marginal folds of the scutellum. The scutum has been generally observed to be comparatively of more width in the females than in the males, as is also the case with the scutellum of the mesothorax. The notal incision lies in the edge of the scutum between the adnotale and the posterior margin of the suralare.

The scutellum is somewhat a rhombus-shaped tergite lying immediately posterior to the mesoscutum in *Scirpophaga nivella* (Plate IX, Fig. 1) and *Chilotræa infuscatellus* (Plate IX, Fig. 5). It approaches a rhombus-shaped structure with its posterior sides distinct but subrounded in case of *Chilo tumidicostalis* (Plate IX, Fig. 2) and *Chilo zonellus* (Plate IX, Fig. 4). The posterior sides are somewhat transversely or horizontally subrounded in *Sesamia inferens* (Plate IX, Fig. 10).

The two anterior sides of the mesoscutellum are formed and bounded by the two arms of the reversed V-shaped ($\wedge$) endotergal ridges of the scutoscutellar suture which lies with its apex directed forward dividing the notum into an anterior scutum and a posterior scutellum. The two posterior sides of the mesoscutellum are formed by the two endotergal ridges which are probably formed by the transcutellar suture and run quite close and above

the intersegmental fold of the antecostal ridge between the scutellum and the metathoracic scutum in case of *Scirpophaga nivella, Chilotræa infuscatellus, C. auricilia* and *Emmalocera depressella.* These ridges are fused with the intersegmental fold itself in case of *Chilo tumidicostalis, C. zonellus* and *Sesamia inferens.*

A post-transcutellar suture forms an internal ridge which cuts through the posterior part of the mesothoracic scutellum, bracing it internally. Posterior to and below the mesoscutellum, behind the internal ridge, lies the post-scutellum which bears the second phragma. This plate represents the second post-notum with which is incorporated the acrotergite of the metathorax. The post-phragma formed between the post-scutellum and the metathoracic scutum is developed to such an extent so as to force the dorsal organs and the alimentary tract into the ventral region of the body cavity in order to pass beneath.

The pleural suture divides the pleuron into an episternum and an epimeron. The episternum in turn is again divided by the anepisternal suture into a dorsal anepisternum and a ventral katepisternum which continues as a narrow pre-coxale and fuses with the furcasternum. The presternum is fused with the basisternum ventrally and the pre-eipsternal suture separates it from the katepisternum. The basal portion of the basealare may be represented by a dorsal fold in the anepisternal margin but the basealare proper is represented by a partially detached, triangular plate lying dorsad of the fold.

The V-shaped epimeron is quite distinct. Its anterior portion is cut off into a somewhat subcircular region by a distinct suture. Shepard (1930) called this as pre-epimeron. The upper portion of the pre-epimeron lies in a cavity, extending forward into the anepisternum while its anterior margin overlies the pleural suture. The narrow and antero-dorsal portion of the epimeron is produced dorsally into a pleural wing process. The epimeron does not form a post-coxale and tapers to a sharp point immediately behind the meron.

The subalare plate lies in the membrane above the epimeron and is invaginated mesally to support the important wing muscles. The second subalare is a small narrow plate and is located behind the subalare. The mesothoracic spiracle (Plate VIII, Figs. 2 and 3) has migrated forward and lies in the pleural membrane of the prothorax caudad of the patagia.

A broad wedge-shaped sclerite inrolled to form a mid-ventral suture resembling an internal keel-shaped ridge represents the basisternum. The furcasternum is a small triangular deeply infolded plate. Its median invagi-

nation forms a pair of broad furcal arms directed slightly caudad. The posterior portions of the epimera are fused with these arms to form a brace across the posterior end of the mesothoracic cavity. There is no visible connection between the furcal arms and the pleural ridge. The structure of the endoskeleton is much complicated by additional invaginations and infoldings and it is rather very difficult to determine this point.

The post-scutellum is a small plate lying posterior to and below the mesoscutellum. It bears the second phragma to which are attached the muscles.

(*c*) *Metathorax* (Plate X, Figs. 1–8).—The Scutum consists of two lateral lobe-like sclerites somewhat triangular in shape with arms almost across in all the Pyralids but in *Sesamia inferens* where the two lobes of the scutum are comparatively less developed, and the metascutellum is also much reduced. The suralares are formed by the margins of the scutum anteriorly which are produced slightly. The two adanales are formed by the produced and flattened posterior margins of the scutum.

The Scutellum is a transverse triangular plate with its apex directed forward and lies across the base of the scutum. It is somewhat helmet-shaped in males of *Scirpophaga nivella* (Plate IX, Fig. 1) and *Chilotræa infuscatellus* (Plate IX, Fig. 5). In females of the latter (Plate X, Fig. 4) it is somewhat a transverse or horizontal reversed boat-shaped structure. It is again somewhat a similar structure in *Chilo tumidicostalis* (Plate X, Fig. 2 and *Chilo zonellus* (Plate X, Fig. 3), *Bissetia steniellus* (Plate X, Fig. 6), and *Emmalocera depressella* (Plate X, Fig. 7). It is comparatively a transverse band or plate in case of *Sesamia inferens* (Plate X, Fig. 8).

The membranous axillary cords are the laterally produced margins of the scutellum and extend to the anal region of the hind wings. The post-scutellum lies immediately behind the scutellum and is represented by a deep invagination forming a large internal phragma for the attachment of the muscles.

Convergent Ridges consist of two vertical ridges formed by the so-called convergent sutures and are very prominent throughout the entire posterior part of the notum and cut through the metascutum and the metascutellum, forming a median area in each case. These vertical ridges have a common transeverse ridge at their base, posteriorly, and mesally. The entire area surrounded by these sutures and the posterior base is comparable to a drinking glass or a flower vase. The posterior transverse ridge of these sutures or the base, as this may be called, has been observed to exhibit various kinds of structures or shapes which appear to be useful in tracing out the evolution

and identification of different species to some extent. The two convergent ridges mentioned above are bent posteriorly to join the transverse median ridge. Collectively these appear to be a single continuous ridge in the form of a broad U or rounded V. These endotergal convergent ridges brace the entire structure of the thorax. Their presence is undescribed by the Lepidopterists in the past. Anteriorly these approach the antecostal ridge of their side which run along the sides of the mesothoracic notum anteriorly. The scutellum is cut apart into three parts by these convergent ridges and one may be mistaken in tracing out its boundaries. The median piece lies in the centre and is the biggest one.

Posterior Median Transverse Ridge is generally bent inward with the two lateral or side arms of the vertical ridges produced and a deep circular concavity (Plate X, Fig. 1) formed anteriorly in *Scirpophaga nivella.* In *Chilo tumidicostalis* (Plate X, Fig. 2) it is somewhat a horizontal and straight ridge, subrounded laterally. It is in continuation with the two lateral vertical ridges, thus completing the base of a flower vase. In *C. zonellus* (Plate X, Fig. 3) it is slightly bent inwards anteriorly and the arms of the two lateral vertical ridges are subrounded. There is a reversed V-shaped concavity formed by the inward bent of the transverse median ridge in case of *Chilotræa infuscatellus* (Plate X, Fig. 4). In *Chilotræa auricilia* (Plate X, Fig. 5) and *Bissetia steniellus* (Plate X, Fig. 6) the inward bent is slightly more than that of *Chilo* species. It appears to be almost similar in these two species except that the right arm or the lobe in the former appears to be slightly longer and thicker than the left one while in the latter, *i.e.*, *Bissetia steniellus* the case is just the reverse, the left arm being slightly longer than the right one. In *Emmalocera depressella*, the right arm is comparatively much reduced while the left arm protrudes posteriorly, thus giving an irregular look of the transverse median ridge (Plate X, Fig. 7). The females of *Emmalocera depressella* have been observed to possess a bowl-shaped concavity on this median transverse ridge as exhibited sometimes by the females of *Chilotræa infuscatellus*. This character should therefore be applied a little carefully in these two species. In *Sesamia inferens* (Plate X, Fig. 8) the median transverse ridge is convex, bulging towards the right side. It is somewhat rounded, a character which separates this noctuid moth from the rest of the *Pyralidæ* under study.

The vertical pleural suture divides the metapleuron into an anterior episternum and a posterior epimeron. The episternum and the basisternum are fused ventrally. The basealare pad or the anterior basealare described by Sheppard (1930) is a weakly sclerotized sub-circular pad situated on the dorsal margin of the episternum.

The metathoracic spiracle is situated beneath the basealare pad and the vertical pleural wing process lies behind the basealare. The wing process, basealare pad and the basealare afford for the attachment of the muscles thus supporting the wings.

The subalare is situated above the deep membranous emargination of the dorsal margin of the epimeron. The posterior region of the epimeron fuses with the latero-ventral extensions of the post-scutellum to form a post-alare bridge. The invaginated post-scutellum merges with the upper portion of the post-alare bridge which is deeply infolded.

The basisternum is represented by the reduced narrow pre-coxale in front of the coxal cavity. The furcasternum consists of two pairs of arms, *i.e.*, the dorsal anterior and posterior pair of furcal arms. The anterior arms are short and stout while the posterior arms are fused with the epimeron ventrally to give rise to a narrow post-coxale, attached to the basal abdominal sternite and are much longer. The furcal arms of the furcasternum are not connected apparently with the pleural ridge which corresponds with the condition existing in the mesothorax.

4. Legs

The legs of these moths have somewhat a similar fundamental structure as described for other Lepidoptera, but for the minor variations here and there.

The coxa of the prothoracic legs in these insects, as usual, is an elongated and cylindrical segment with a broad base tapering towards the distal end. Proximally it articulates with the trochantin in all cases and bears a concavity at its ventral surface (Plate XIII, Figs. 1–8). In case of mesothoracic and metathoracic legs the coxa is immovably united with the pleura. A basicostal suture divides the coxæ of the meso- and metathoracic legs into an anterior eucoxæ and the posterior mera, these being produced distally into trochantifers articulate freely with the condyles of the trochanters. The basicostal suture in these cases separates a triangular plate from the eucoxa at the base of the coxa lying between the katepisternum and the meron. Snodgrass (1909) calls this plate the trochantin but Sheppard (1930) calls it as epicoxal piece. The author agrees with the latter author and the structure in question may not be called as the trochantin.

Trochanter—The trochanters of all the legs are sub-globose and of about equal length. Distally these are immovably attached to the femur while the proximal articulation is between the condyles and the trochantifers.

Femur—The posterior margin of the femur of the prothoracic leg is widely rounded proximally but gradually tapers towards the distal end, thus providing for the accommodation of the epiphyses when the femur and tibia are folded together. The femora are clothed with hairs. The femora of the prothoracic legs are nearly $1\frac{1}{2}$ times the length of the femora of the meso and metathoracic legs in *Scirpophaga nivella*. These are either equal to or a little less than the meso and metathoracic femore in case of *Chilotræa infuscatellus*, *C. auricilia*, *Chilo zonellus* and *C. tumidicostalis*. The femora of the prothoracic legs are less than the length of the meso- and metathoracic femore in *Bissetia steniellus* as is the case in *Sesamia inferens* and *Emmalocera depressella*.

Tibia—The tibia of the prothoracic legs of these moths is short and stout and bears a pair of lamellate spurs or epiphyses on the inner surface. The tibia of the meso- and metathoracic legs are $2\frac{1}{2}$ to 3 times the length of the tibia of the prothoracic leg in case of *Scirpophaga nivella* (Plate XIV, Fig. 1). Similarly it is 3 to $3\frac{1}{2}$ times in case of *Chilotræa auricilia*, *C. infuscatellus* and *Bissetia steniellus* and 3 times or a little less in case of *Chilo zonellus*, *C. tumidicostalis* and *Sesamia inferens*. The mesothoracic tibia is rather slender, but widens towards the distal end which is armed with a pair of stout spurs in all the species. The inner spur is about half of the length of the outer spur, which in turn is about half as long as the tibia. Each metathoracic tibia is provided with two pairs of spurs, one at the distal end and the other at about $\frac{2}{3}$rd the length of the tibia. The longer spur of each pair is on the outer side. It is interesting to note that all the tibiæ are provided with scattered irregular short stout setæ and sensory pits all over the surface in case of *Sesamia inferens* (Plate XIV, Fig. 9) a character which separates the species from the other species of sugarcane moth borers described in the present paper.

Tarsus—The tarsi of all the legs are composed of five joints, the basitarsus being the largest in all the species. The segments become progressively shorter and shortest is the distitarsus. The joints of the tarsi are covered with spines, hairs and bristles.

Pretarsus—A claw or the so-called pretarsus (Plate XII, Fig. 8) is present at the tip of the distitarsus of each leg. It is composed of two large curved ungues which articulate with the dorsal margin of the distitarsus by means of unguifers or the small hooks. The base of the claw bears the membranous pulvilli. Proximad of the base of the claw there is a broad sclerite, the basal portion of which apparently represents the planta, with a median hair-like distal elongation which is probably the empodium. A small membranous

area above the empodium which bears a tiny sclerotized orbicula probably represents the arolium. The sclerotized unguitracter is partially withdrawn into the distitarsus and is separated from the base of the planta.

TABLE I

Showing the diagnostic characters in the thorax

Species	Median suture forming the median carina	Shape and structure of the posterior transverse ridge	Rows of spines on the legs
1. *S. nivella*	Well developed	Generally bent inward	Absent
2. *C. tumidicostalis*	Obliterated	Horizontal and straight ridge, sub-rounded laterally. The entire continuation with the vertical ridges gives an appearance of the base of a flower vase	Absent
3. *C. zonellus*	Obliterated	Slightly bent inward anteriorly with two arms of the vertical ridges sub-rounded	Absent
4. *C. infuscatellus*	Present	Inward bent of the ridge quite prominent and in the form of a reversed V-shaped concavity	Absent
5. *C. auricilia*	Obliterated	Slight inward bent divides the ridge into two halves as usual but the right portion is slightly longer and thicker than the left one	Absent
6. *B. steniellus*	Absent	Just the reverse as in *C. auricilia*. Here the left portion is longer than the right one	Absent
7. *E. depressella*	Prominent	Inward bent slight. The right arm comparatively much reduced and the left one protrudes posteriorly in certain cases	Absent
8. *S. inferens*	Absent	Convex, bulging towards the right side and is somewhat rounded	Present

N.B.—The last mentioned character, *i.e.*, the presence of the spines on the surface of the legs is a very reliable character to separate the Noctuids from Pyralids.

5. DIAGNOSTIC CHARACTERS

Inspite of the fact that these sugarcane moth borers exhibit a remarkable constancy as regards their fundamental structure of the thorax is concerned, it has been possible to ascertain some of the characters which are quite useful in their proper identification and tracing out the trend of evolution in these insects. Such important characters have already been mentioned in the text and are also depicted in Table I showing only the diagnostic characters in the thorax of different species. These are based on the modifications of the median transverse ridge, presence or absence of the median carina and the presence of certain rows of spines on the legs of these insects. The presence of such spines is found in the noctuids only and is therefore a very reliable character to separate any noctuid sugarcane moth borer from the other Pyralid moth borer of sugarcane.

6. SUMMARY AND CONCLUSIONS

1. The anatomy of the adult thorax in eight different species of sugarcane moth borers, *viz.*, *S. nivella*, *Chilo tumidicostalis*, *Chilo zonellus*, *Chilotræa infuscatellus*, *C. auricilia*, *Bissetia steniellus*, *Emmalocera depressella* and *Sesamia inferens* is described in detail.

2. The diagnostic characters such as the presence or absence of the median carina, modifications of the median transverse ridge and the presence of the spines on the legs have been observed to be quite reliable characters for the proper identification of these insects.

3. Finally a table showing various modifications of different important structures in different species has been furnished.

7. ACKNOWLEDGMENTS

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Explanation of Plates

Plate VIII. *Cervix*

Figs. 1 and 2. *Scirpophaga nivella.*
Fig. 3. *Chilo tumidicostalis.*
Fig. 4. *Chilo zonellus.*
Fig. 5. *Chilotræa infuscatellus.*
Fig. 6. *Chilotræa auricilia.*
Fig. 7. *Bissetia steniellus.*
Fig. 8. *Emmalocera depressella.*
Fig. 9. *Sesamia inferens.*

Plate IX. *Thorax (Full view) in the males*

Fig. 1. *Scirpophaga nivella.*
Fig. 2. *Chilo tumidicostalis.*
Fig. 3. *Chilo tumidicostalis.*
Fig. 4. *Chilo zonellus.*
Fig. 5. *Chilotræa infuscatellus.*
Fig. 6. *Chilotræa infuscatellus.*
Fig. 7. *Chilotræa auricilia.*
Fig. 8. *Bissetia steniellus.*
Fig. 9. *Emmalocera depressella.*
Fig. 10. *Sesamia inferens.*

Plate X. *Thorax (Complete view) Dorsal*

Fig. 1. *Scirpophaga nivella.*
Fig. 2. *Chilo tumidicostalis.*
Fig. 3. *Chilo zonellus.*
Fig. 4. *Chilotræa infuscatellus.*
Fig. 5. *Chilotræa auricilia.*
Fig. 6. *Bissetia steniellus.*
Fig. 7. *Emmalocera depressella.*
Fig. 8. *Sesamia inferens.*

PLATE XI. *Thorax (Ventral view)*

FIG. 1. *Scirpophaga nivella.*
FIG. 2. *Chilo tumidicostalis.*
FIG. 3. *Chilo zonellus.*
FIG. 4. *Chilotræa infuscatellus.*
FIG. 5. *Chilotræa auricilia.*
FIG. 6. *Bissetia steniellus.*
FIG. 7. *Emmalocera depressella.*
FIG. 8. *Sesamia inferens.*

PLATE XII. *Thorax (Lateral view)*

FIG. 1. *Scirpophaga nivella.*
FIG. 2. *Chilo tumidicostalis.*
FIG. 3. *Chilo zonellus.*
FIG. 4. *Chilotræa infuscatellus.*
FIG. 5. *Chilotræa auricilia.*
FIG. 6. *Bissetia steniellus.*
FIG. 7. *Emmalocera depressella.*
FIG. 8. Pretarsus *in Chilo tumidicostalis.*

PLATE XIII. *Prothoracic leg*

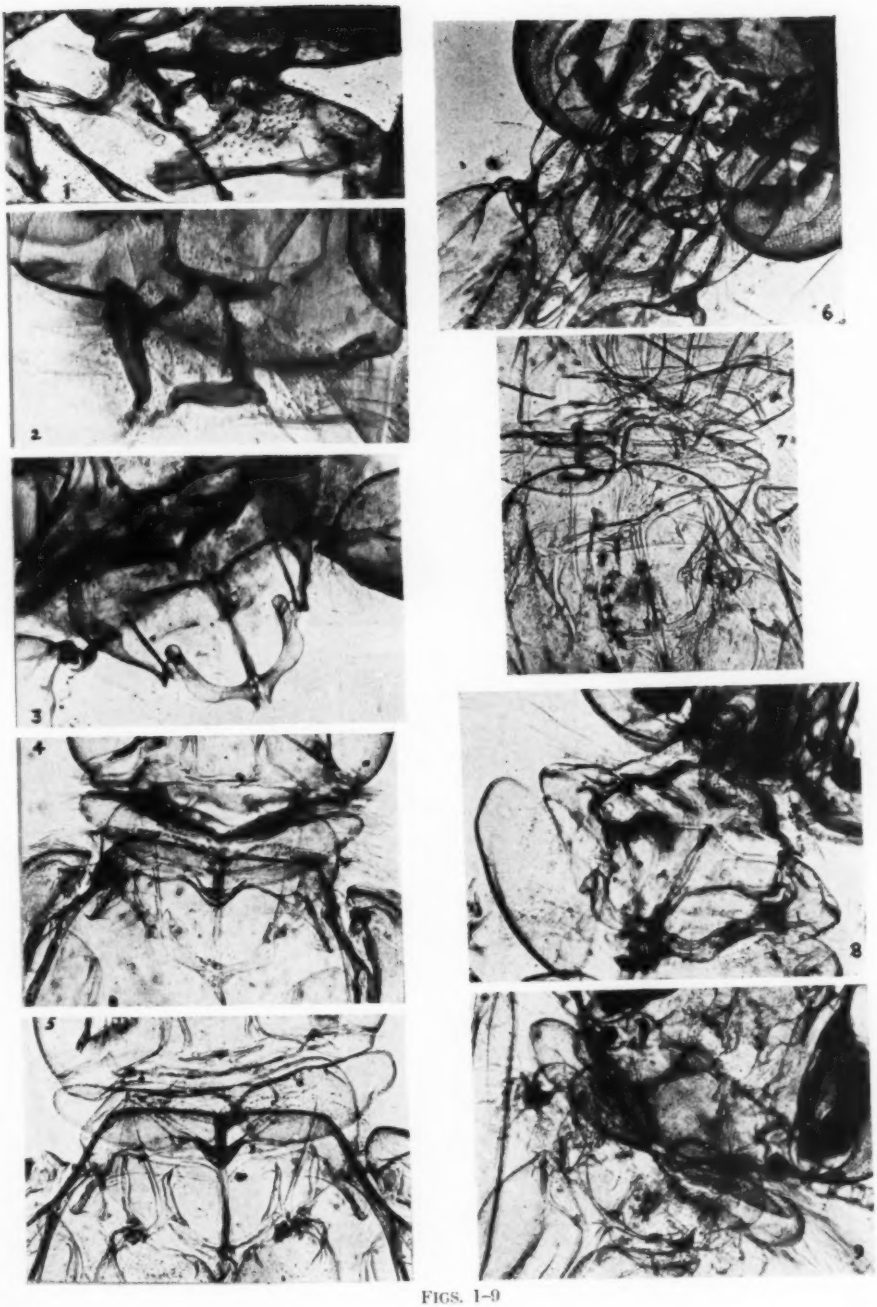
FIG. 1. *Scirpophaga nivella.*
FIG. 2. *Chilo tumidicostalis.*
FIG. 3. *Chilo zonellus.*
FIG. 4. *Chilotræa infuscatellus.*
FIG. 5. *Chilotræa auricilia.*
FIG. 6. *Bissetia steniellus.*
FIG. 7. *Emmalocera depressella.*
FIG. 8. *Sesamia inferens.*

PLATE XIV. *Mesothoracic leg*

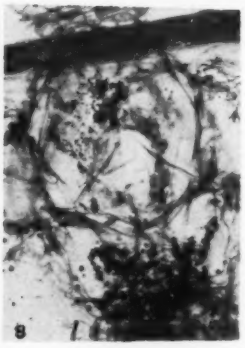
FIG. 1. *Scirpophaga nivella.*
FIG. 2. *Chilo tumidicostalis.*
FIG. 3. *Chilo zonellus.*
FIG. 4. *Chilotræa infuscatellus.*
FIG. 5. *Chilotræa auricilia.*
FIG. 6. *Bissetia steniellus.*
FIG. 7. *Emmalocera depressella.*
FIG. 8. *Sesamia inferens.*
FIG. 9. *Sesamia inferens* (Enlarged view of the leg showing the spines and the sensory pits).

PLATE XV. *Metathoracic leg*

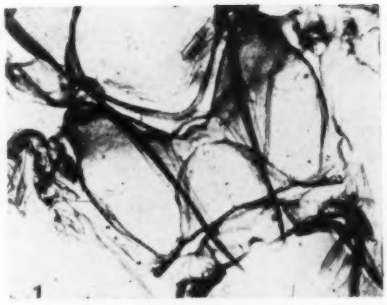
FIG. 1. *Scirpophaga nivella.*
FIG. 2. *Chilo tumidicostalis.*
FIG. 3. *Chilo zonellus.*
FIG. 4. *Chilotræa infuscatellus.*
FIG. 5. *Chilotræa auricilia.*
FIG. 6. *Bissetia steniellus.*
FIG. 7. *Emmalocera depressella.*
FIG. 8. *Sesamia inferens.*

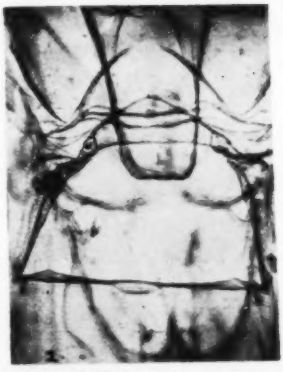


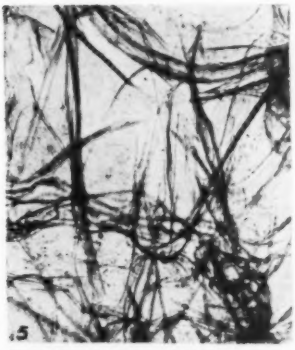
FIGS. 1–9

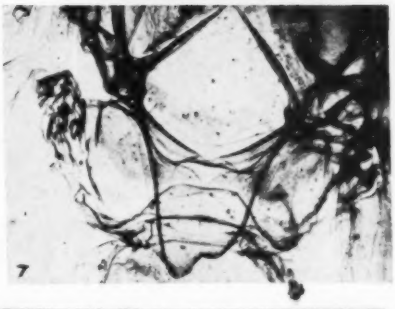


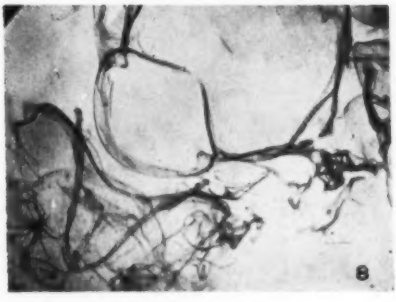
FIGS. 1–11











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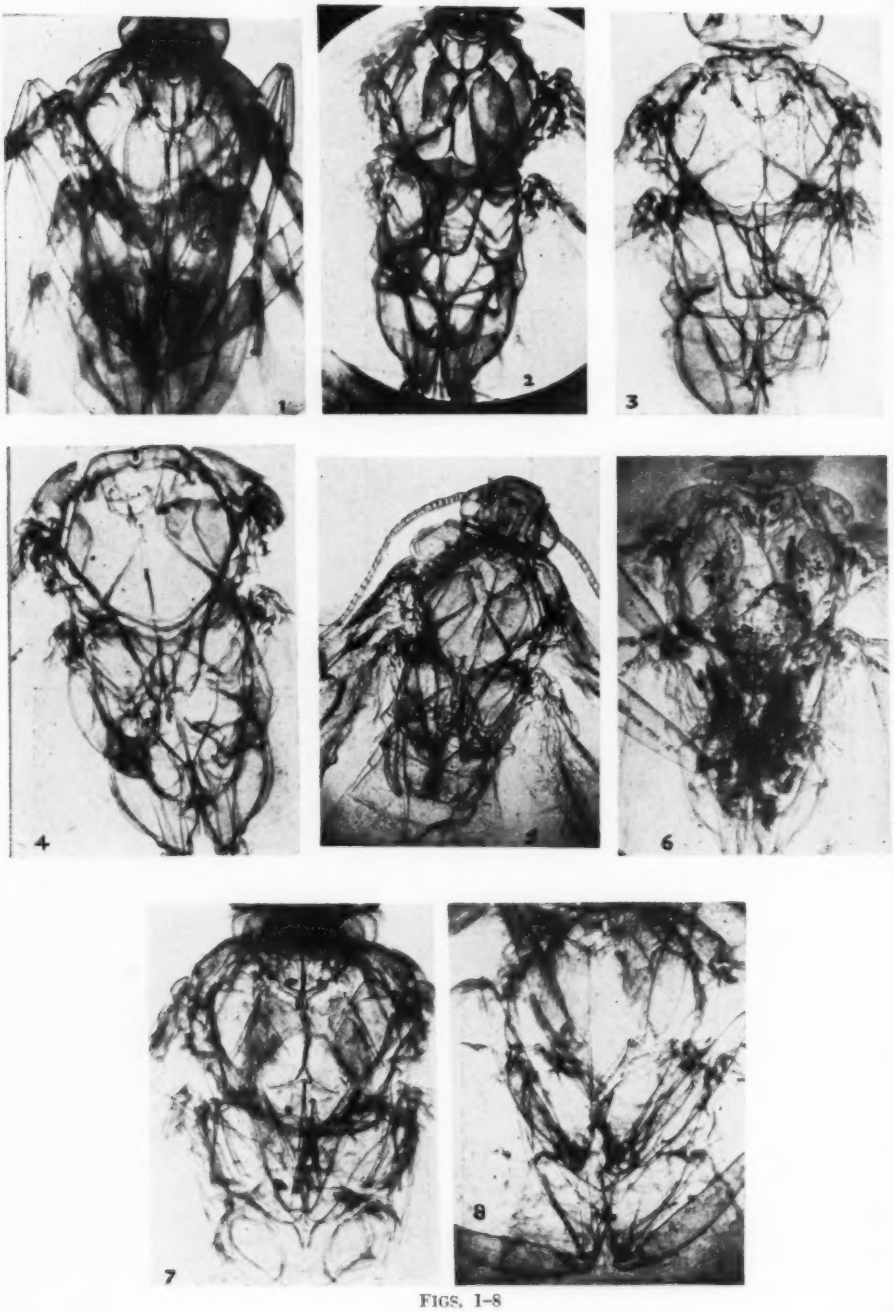

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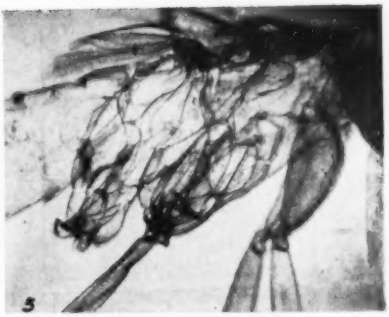
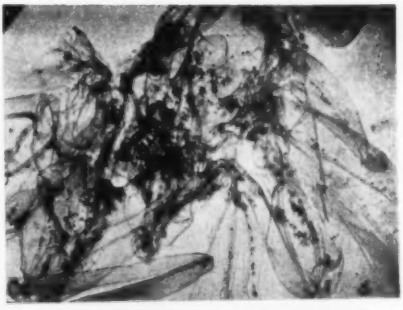
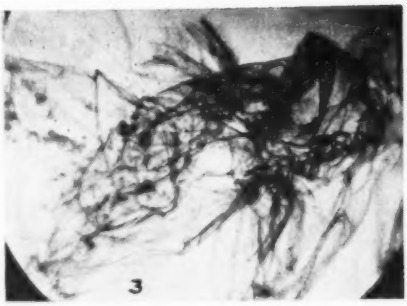
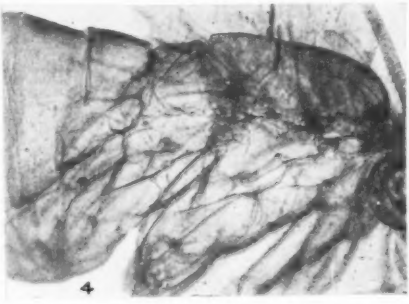
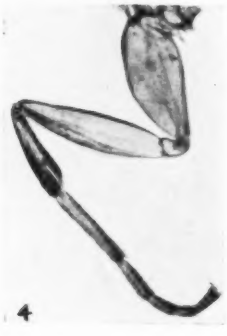








FIGS. 1–8



FIGS. 1-8

FIGS. 1–9

FIGS. 1–8

STUDIES ON THE ANATOMY OF THE SUGARCANE MOTH BORERS

VI. Comparative Study of the Female Genitalia

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1. Introduction

The study of the genital armature in insects is obviously the most important item in systematic work and has evoked considerable interest in modern taxonomy because of its definite and reliable bearings on the identification and classification of different groups of insects. Tams (1926) stated that the genital armature in many groups of insects both in the male and the female presents characters valuable in the identification of different species.

The study of the female genitalia in Lepidoptera has been far more neglected in comparison to that of the male genitalia possibly because of the fact that the female armature was considered comparatively simple (McDonough, 1911).

Although the genital armature of different sugarcane moth borers (Lepidoptera) has been studied by quite a few workers in the past, the fact remains that very little attention has been paid to the female genitalia. Bisset (1939) proposed the name *Chilo trypetes* (*Bissetia steniellus*) for the new pyralid borer from Gurdaspur (Panjab) and compared the male and female genitalia of three different species of the genus *Chilo*, *viz.*, *Chilo suppressalis*, *C. zonellus* and *C. trypetes*, with the help of their illustrations. He did not, however, describe the female genitalia of these species. Gupta (1940) studied the genitalia of four different species of sugarcane moth borers, *viz.*, *Chilotræa infuscatellus*, *C. auricilia*, *Proceras indicus* and *Chilo zonellus*, but did not describe these structures in detail. His descriptions were further not supported by illustrations. Trehan and Butani (1950) discussed the systematic position of *Chilo zonellus* and *C. simplex* by illustrating the difference between the male genitalia of these species. They did not compare the female genitalia thus leaving a considerable gap in knowledge. Kapur (1950) considered mainly the male genitalia and other characters in connection with his proposed revision of the taxonomy of crambinid moth borers of sugarcane. He did not describe the detailed structure of the ovipositor in case of *Chilotræa infuscatellus*, *C. auricilia* and *Chilo tumidicostalis*. The female genital openings also received inadequate attention. Besides his study was confined to a single subfamily, Crambinæ. In the present paper, three subfamilies of the family *Pyralidæ* and another subfamily *Agrotidæ* of the family *Noctuidæ*, have been presented, thus including another three important species of sugarcane moth borers, *viz.*, *Scirpophaga nivella* (*Schœnobiinæ-Pyralidæ*), *Emmalocera depressella* (*Anerastinæ-Pyralidæ*) and *Sesamia inferens* (*Agrotidæ-Noctuidæ*). Narayanan and Venkatraman (1951) confirmed the identity and synonymy of *Scirpophaga nivella* and *S. monostigma* by comparing their male genitalia but they too did not attach any importance to the female genitalia which according to Kapur (*loc. cit.*) are more helpful in separating out the different species of the same genus. Krishnamurti and Usman (1952) have added much to knowledge of the morphology of *Sesamia inferens* but they also have ignored illustrations of female genitalia of the species. Puri (1957*a*) described and illustrated the male and female genitalia of the sugarcane stem borer *Chilo tumidicostalis*. He (1957*b*) also described the morphology of the female genital chamber in *Emmalocera depressella*, root borer moth of sugarcane.

The present work was undertaken with a view to bridging the existing gaps in knowledge with respect to the detailed anatomy of female genitalia in eight species of sugarcane moth borers.

2. Material and Methods

The moths of seven different species, *viz.*, *Scirpophaga nivella*, *Chilo tumidicostalis*, *C. zonellus*, *Chilotræa infuscatellus*, *C. auricilia*, *Emmalocera depressella* and *Sesamia inferens* were reared in the laboratory at Pusa, Bihar from field collections of larvæ and pupæ. Those of *Bissetia steniellus* were imported from Uttar Pradesh and Panjab for the present work.

Permanent slides of the entire insects as well as those of the abdomen alone were prepared by treating the specimens with 10 to 15% KOH solution, washing them under a current of water for about 8 hours, dehydrating, clearing in carbo-turpentine, turpentine, cedar wood oil and finally mounting them in turpentine canada balsam. The specimens were also stained with eosin in 90% alcohol before clearing them in carbo-turpentine or pure turpentine as described by Puri (1954). This method proved quite satisfactory. The micro-photographs reproduced in the present paper were taken directly from slides of the various structures duly stained.

3. Description of the Various Parts

(*a*) *Structure of the Abdomen*

The abdomen in females is generally elongate, with a comparatively thick and less tapering abdomen than that of males. It is composed of ten segments. The last three segments, *viz.*, 8th, 9th and 10th are usually modified to form the genitalia in these female moths but it has been observed that the 7th segment may also take part in such formations in particular cases like that of *Emmalocera depressella* where the folds of the genital opening or the ostium bursæ do extend upto the 7th abdominal segment. The last two or three segments are usually retractible within the last visible segment. There are only seven apparent segments in the female abdomen of these insects. The sclerotized portions of the unmodified abdominal segments consist of a tergite and a sternite. These are separated by a membranous pleural region bearing the seven abdominal spiracles. The segments are connected by conjunctival membranes and each tergite and sternite normally overlaps the following tergite and sternite. A narrow acrotergite is present in the tergites of all the unmodified segments except the first. The internal antecosta formed by the antecostal suture bears the principal muscles. The 8th abdominal spiracle present in the larval stage is obliterated but its remanents may be observed in the freshly hatched moths. The entire surface of the abdomen is covered with thick scales and hairs.

The general description of the tergites and the sternites of the abdomen is somewhat similar to that given by Madden (1944). The tympana are a pair of vesicles which are sac-like or bladder-like structures closely associated with the first pair of abdominal spiracles on each side, extending upto the 4th abdominal segment in some cases. These are well developed and quite prominent structures, believed to be auditory in function (Imms, 1951), and are innervated from the last thoracic ganglion.

(*b*) *Female Genitalia*

The study of the genitalia in these insects is possible only in denuded specimens made transparent for this purpose, because these are always concealed by a thick clothing of hairs and scales. The female genitalia in these insects are usually of ditrysian type quite common in most of the Lepidoptera, as described by Tuxen (1956). In this type of genitalia the two reproductive openings, *viz.*, the ostium oviductus and the ostium bursæ are widely separated.

(i) *Ostium oviductus and the ovipositor.*—The ostium oviductus opens in the last or the 10th segment cephalad and ventrad of the anus. Both ostium oviductus and anus are between the two lobes of the false ovipositor or the papillæ anales. The ovipositor lobes are usually elongated so as to serve as an extensible ovipositor. These papillæ anales are heavily sclerotized in all these moths, being in the form of an elongated plate. The papillæ anales are united or fused so as to appear an elongated plate-like ovipositor in almost all the Pyralids but in case of *Sesamia inferens* (*Noctuidæ*), the two lobes are partially fused and appear to be separated out as shown (Plate XVIII, Fig. 8). The papillæ anales of the false ovipositor are modified differently in different species of these sugarcane moth borers which are typical of the different species. It is somewhat betel-shaped in *Scirpophaga nivella* (Plate XVIII, Fig. 1). It appears to be somewhat like a slipper sole or paramœcium in case of *Chilo tumidicostalis* (Plate XVIII, Fig. 2). It is somewhat flat in the middle and tapering towards both the ends just like a liver fluke in *Chilo zonellus* (Plate XVIII, Fig. 3). It is interesting to note that the two lobes or the papillæ anales do not appear to be separated apart in *Chilo* species. In *Chilotræa infuscatellus* (Plate XVIII, Fig. 4) there is a notch or a small bowl-shaped concavity at the anterior end of the fused false ovipositor which is situated in the middle of the anterior ends of the two lobes. The entire ovipositor appears to be like a slipper with a flat toe. It is comparatively flat and greatly sclerotized plate-like structure in *Chilotræa auricilia* (Plate XVIII, Fig. 5). The two lobes or the papillæ anales are almost completely fused to form a single elongated plate-like ovipositor in *Bissetia steniellus* (Plate XVIII, Fig. 6) which in the lateral view gives a side view of the funnel. In

Emmalocera depressella (Plate XVIII, Fig. 7) the anterior notch or the concavity noticed in case of *Chilotræa infuscatellus* also persists but the shape of the ovipositor is entirely different. It appears to be somewhat like the blade of a kudali meant for hoeing the potatoes. In *Sesamia inferens* (Plate XVIII, Fig. 8) the two lobes of the ovipositor are fused anteriorly but appear to be separated out posteriorly. These collectively appear to be like a flat and elongated reddish root whose two halves have been separated cut posteriorly with a mid-ventral cut or line, in between which lie the anus and the ductus oviductus. All the ovipositors of these insects are covered with hairs and scales.

(ii) *The genital chamber and the ostium bursæ.*—The genital chamber or the sinus vaginalis occurs mid-ventrally caudad of the 7th segment or on the 7th to 8th segment formed more or less by deep inflexions of the body-wall in most of the species, but in *Emmalocera depressella* the genital chamber has been observed to be located laterally (Plate XVI, Fig. 9). The genital chamber is usually a mid-ventral pocket or fold surrounded by the genital plate from where opens the receptive opening or the ostium bursæ leading into the ductus bursæ and the corpus bursæ, all the three comprising the bursa copulatrix. There have been observed to be two lateral pouches one functional and the other non-functional in *Emmalocera depressella* which collectively form the genital chamber. The location and the shape of the genital openings or the ostium vaginalis along with their genital plates are of great taxonomic value and quite typical of the species even in the same subfamily. In *Scirpophaga nivella* the circular or the oval-shaped genital plate surrounds the oval ostium vaginalis (Plate XVII, Fig. 1). In *Chilo tumidicostalis* (Plate XVII, Fig. 2) the ostium bursæ or the genital opening of the bursa copulatrix lies on a heavily chitinized oval plate. Within this oval plate, the genital opening proper is guarded laterally by two strongly chitinized sword-shaped arms with their apices directed forward. These arms are supported posteriorly on a thick transverse chitinized plate bounding the genital opening posteriorly. These arms probably function as a guide for tracing out the genital opening for the penis of the male during the process of copulation. The genital opening is in the form of a folded pocket in the upper half, in between the two arms, and sunk in the median oval plate. The rim of the external oval plate or the genital plate is provided with a heavily chitinized band. The posterior plate supporting the two sword-shaped arms, guarding the genital opening, joins the rim of the oval plate posteriorly. The ostium bursæ or the genital opening in *Chilo zonellus* (Plate XVII, Fig. 3) is in the form of an inverted funnel lying in between the two lobes of the chitinous shield posteriorly. The chitinous shield or the genital plate as it may be called,

is divided distally or posteriorly into two subrounded halves with prominent, irregular, longitudinal wrinkles. The genital opening or the ostium bursæ is somewhat rhombus-shaped. In *Chilotræa infuscatellus* (Plate XVII, Fig. 4) the genital opening is covered by a strongly chitinized plate arising from the lower wall of the opening and divided posteriorly into two lobes which give a triangular appearance enclosing the slit-like longitudinal genital opening in between them. The genital opening is widely tubular in case of *C. auricilia* (Plate XVII, Fig. 5). The plate encircling the genital opening in this species is a rounded chitinized plate with irregular slanting and transverse wrinkles. The genital opening is guarded by deep longitudinal invagination arising posteriorly from the plate. The entire plate encircling the genital opening gives the appearance of hanging weight of a gas plant. The genital opening in case of *Bissetia steniellus* (Plate XVII, Fig. 6) opens into the genital chamber or pouch, as it may be called, and is guarded by the two lateral folds or walls of the genital pouch. Since the opening is located internally in the pouch, it is rather difficult to describe its exact shape but it appears to be somewhat an oval and longitudinal opening. The genital pouch is a median ventral invaginated folded pocket. The genital chamber of *Emmalocera depressella* (Plate XVII, Fig. 7) has been described and illustrated by Puri (1957) who stated that the genital chamber of this species constitutes an external pocket of the body-wall with a large median internal pouch and two lateral pouches. One of these, *viz.*, the left one becomes the functional opening of the vagina for copulatory purposes and is sunk in the folded wall. The other lateral pouch is non-functional and seems to have no connection with the vagina. The median pouch is functionally the vagina but is the part of the chamber. The genital opening in this case is therefore an internal opening. In *Sesamia inferens* (Plate XVII, Fig. 8) the ring around the genital opening forms a longitudinal tube protruding ventrad for some distance in between the anterior and posterior parts of the sterigma or the genital plate. Tuxen (1956) calls the anterior parts as the lamella antevaginalis and the posterior parts as the lamella postvaginalis. The genital opening is joined with the anterior parts of the lamella antevaginalis in the form of mid-ventral longitudinal deep fold. The structures of the genital chamber or the pouch, the sterigma or the genital plate, and the shape of the genital opening proper throw much light on the course of evolution in these insects which need still further extensive study of the allied forms in order to ascertain the lines of evolution.

(iii) *The ductus bursæ and the corpus bursæ.*—Both the ductus bursæ and the corpus bursæ may be variously sclerotized and are well differentiated, the former being a long tube and the latter a sac-like structure. The ductus

bursæ may be a short or a long tube leading to the sac-like corpus bursæ which may have different shapes in different species. It is almost a flattened globular rounded ball-like structure in *S. nivella* (Plate XVI, Figs. 1 and 2). In *Chilo tumidicostalis* (Plate XVI, Fig. 3) it is tubular or sausage-like, three to four times as along as wide. Signum, the usual spined or scobinate structures in the walls of the corpus bursæ of these two species, is absent. The signa too are enormously variable in size and shape and supposed to be connected with the breaking up of the spermatophores as described by Tuxen (1956). The corpus bursæ in *Chilo zonellus* (Plate XVI, Fig. 4) is sub-globular to oval and as long as wide. It is provided with a small spined signum. In *Chilotræa infuscatellus* (Plate XVI, Figs. 5 and 6) the corpus bursæ resembles the head of a closed mushroom and is about 1½ to 2 times as long as wide. It is provided with a small spined signum, which is comparatively more prominent than that of *C. zonellus*. In *Chilotræa auricilia* (Plate XVI, Fig. 7) the corpus bursæ is sub-rounded to oval without any signum. A very prominent, highly chitinized folded sac-like corpus bursæ may be observed in *Bissetia steniellus* (Plate XVI, Fig. 8), which is provided with a signum composed of fork organs. In *Emmalocera depressella* (Plate XVI, Fig. 9) the corpus bursæ is a bag-like structure provided with a strongly chitinized signum comprised of a capitulum with prickled body. The neck and mouth of the bag-like corpus bursæ may be called the appendix bursæ. In *Sesamia inferens* (Plate XVI, Fig. 10) the corpus bursæ is an elongated bladder-like sac provided with a sharp conical appendix bursæ and a spined signum. The corpus bursæ is like a flageolet instrument of the snake charmers. The signum may be trident or band-like.

(iv) *The ductus seminalis.*—The ductus seminalis arises from the corpus bursæ direct in case of *S. nivella* and joins the spermathecæ or the spermathecal sacs, by bifurcating into two parts (Plate XVI, Figs. 1 and 2). The ductus seminalis in other moth borers under study also seem to arise either from the ductus bursæ or from the corpus bursæ.

4. Diagnostic Characters

The important characters in the female genitalia of different species of sugarcane moth borers under study have been observed to be of great systematic value and have already been described and discussed above. These have been described and compared in Table I.

5. Summary and Conclusions

1. The female copulatory organs in eight different species of sugarcane moth borers, *viz.*, *Scirpophaga nivella*, *Chilo tumidicostalis*, *Chilo*

TABLE I. *Showing the diagnostic features in different species*

Species \ Characters	Structure of the Ovipositor (Papillæ anales)	Position and structure of the Genital chamber and the ostium bursæ	Structure of the Corpus bursæ with or without signum	Form of signum
Scirpophaga nivella ..	Betel-shaped	Ventral like a mid-ventral pocket. Ostium bursæ surrounded by the wrinkled oval or rounded genital plate	A flattened globular rounded ball-like	Indistinguishable
Chilo tumidicostalis ..	Somewhat like the sole of a slipper or a paramœcium	Like a mid-ventral pocket surrounded by a heavily chitinized oval plate. Ostium bursæ guarded by two sword-shaped arms with their apices directed forward. It is further sunk in the median oval plate	Tubular or sausage-like 3 to 4 times as long as wide	Absent
Chilo zonellus ..	Flat in the middle and subrounded tapering towards both the ends	Mid-ventral pocket with a chitinous shield or the genital plate with irregular genital wrinkles. Ostium bursæ in the form of an inverted funnel somewhat rhombus-shaped	Sub-globular to oval, as long as wide. Signum present	Spinal or spined
Chilotræa infuscatellus ..	Like a slipper with a flat toe and a notch or a bowl-shaped concavity in the middle at the anterior end of the ovipositor	Mid-ventral pocket triangular in shape. Ostium bursæ in the form of a longitudinal slit in between the two lobes	Somewhat like the top or head of a mushroom 1½ to 2 times as long as wide. Signum present	Spined
Chilotræa auricilia ..	A flat plate-like structure almost completely fused and without a well marked concavity	Mid-ventral pocket with a rounded chitinized plate with irregular transverse and slanting wrinkles. The entire plate encircling the ostium bursæ gives an appearance of hanging weight of a gas plant. The ostium bursæ is widely tubular guarded by deep longitudinal invaginations	Subrounded to oval	Absent
Bissetia steniellus ..	Somewhat like the mouth of a funnel. It appears to be a single elongated plate	Mid-ventral invaginated folded pocket. Ostium bursæ sunk into the genital chamber. It appears to be somewhat longitudinally oval	Sac-like. Signum very prominent	In the form of fork-organs
Emmalocera depressella	Somewhat like the blade of a *Kudali* for hoeing the potatoes. The anterior notch prominent	Lateral infolded pocket composed of a large median internal pouch and two lateral pouches. The left one provides for the ostium bursæ sunk in the folded wall while the other one is non-functional. The median pouch is functionally the vagina	Bag-like with appendix bursæ. Signum present	In the form of a capitulum with prickled body
Sesamia inferens ..	Thick, heavily sclerotised like the reddish root. The papillæ anales appear to be fused anteriorly and separate posteriorly	Mid-ventral pocket. The genital plate or the sterigma is composed of the lamella antevaginalis and the lamella postvaginalis. The genital opening is longitudinal and tubular lying in between the two lamellæ	Elongated bladder-like sac with a sharp conical appendix bursæ. It looks like the flageolet instrument of the snake charmers	In the form of spine

zonellus, Chilotræa infuscatellus, C. auricilia, Bissetia steniellus, Emmalocera depressella and *Sesamia inferens* have been described in detail.

2. A detailed description of the structure of the ovipositor or the papillæ anales, which is of great taxonomic significance in the order Lepidoptera has been given in these species.

3. The location and structure of the genital opening or the ostium bursæ are also of great taxonomic value and help in the identification of these species. Their structures have been described and illustrated in detail.

4. Signa of the various moths have been observed to possess various shapes and are of great value in the proper identification of different species. Their presence or absence is also a useful character in separating out the two species of the same genus and needs further investigations in detail.

5. The general plan of female genitalia in all these insects is of ditrysian type where there are two different openings one for copulation and the other for laying eggs, the former being known as the ostium bursæ while the latter is the oviporus of the false ovipositor in Lepidoptera. The ovipositor of these insects is composed of two lobes, the papillæ anales. These may be fused partially or completely as the case may be but the two lobes together possess different shapes which are of great toxonomic significance.

6. It has been observed that the ductus seminalis arises from the corpus bursæ in case of *Scirpophaga nivella* and joins the spermathecæ after bifurcating into two ducts.

7. Finally a table showing diagnostic features based on the characters of the female genital organs has been furnished.

6. Acknowledgments

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8. EXPLANATION OF PLATES

PLATE XVI. *Female Genitalia (Complete view)*

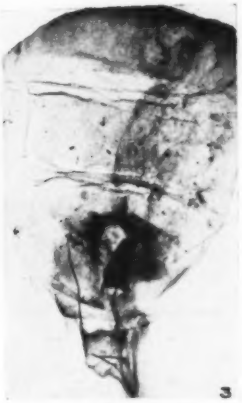
1. *Scirpophaga nivella.*
2. do. side view.
3. *Chilo tumidicostalis.*
4. *Chilo zonellus.*
5 and 6. *Chilotræa infuscatellus.*
7. *Chilotræa auricilia.*
8. *Bissetia steniellus.*
9. *Emmalocera depressella.*
10. *Sesamia inferens.*

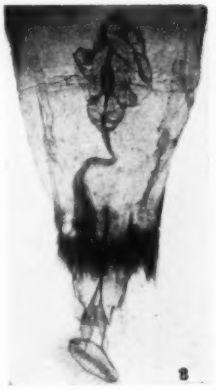
PLATE XVII. *The genital chamber and the ostium bursæ (Enlarged)*

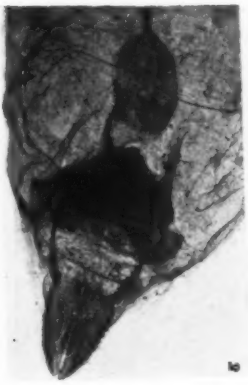
1. *Scirpophaga nivella.*
2. *Chilo tumidicostalis.*
3. *C. zonellus.*
4. *Chilotræa infuscatellus.*
5. *Chilotræa auricilia.*
6. *Bissetia steniellus.*
7. *Emmalocera depressella.*
8. *Sesamia inferens.*

PLATE XVIII. *Ostium oviductus and the ovipositor (Enlarged)*

1. *Scirpophaga nivella.*
2. *Chilo tumidicostalis.*
3. *C. zonellus.*
4. *Chilotræa infuscatellus.*
5. *C. auricilia.*
6. *Bissetia steniellus.*
7. *Emmalocera depressella.*
8. *Sesamia inferens.*







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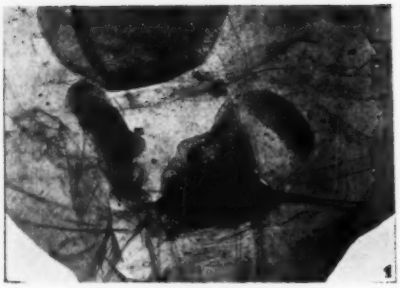
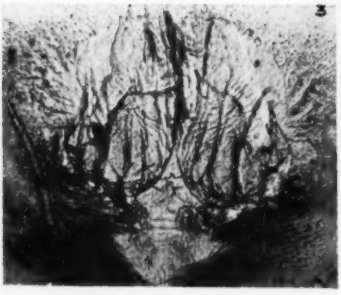
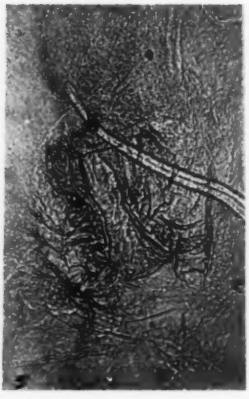
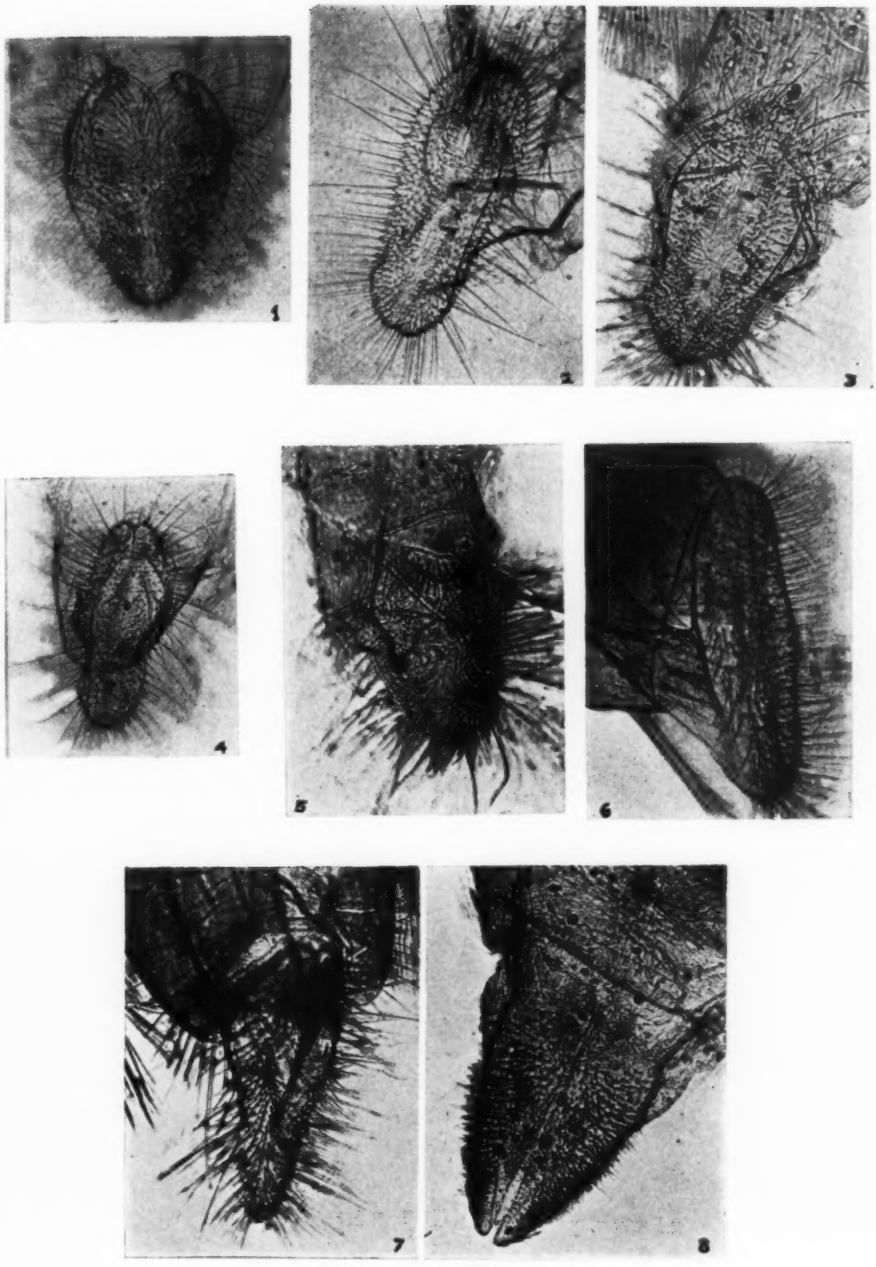






FIGS. 1–8



FIGS. 1–8

STUDIES IN EMBRYOLOGY OF *CYPERUS TEGETUM* ROXB.

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Introduction

One of the most interesting points in the embryology of the Cyperaceæ is the development of the pollen grains. In contrast to all other angiosperms, only one microspore in a tetrad comes to maturity while the other three degenerate. The nuclei of the degenerating microspores besides, are enclosed within the wall of the functioning pollen grain, but it is doubtful whether or not the nucleus of the functioning microspore is separated from the remaining nuclei by a wall. Further, it is debatable whether the degenerating nuclei themselves are separated from each other by distinct septa. According to Piech (1928) the nucleus of the functioning microspore in *Scirpus paluster* is not separated from the remaining three nuclei by a cell-wall. Neither any cell-wall exists delimiting the three non-functioning nuclei. Tanaka's (1939 *b*) observations on *Fimbristylis cericea* agree with those of Piech (1928). In his later work Tanaka (1939 *a* and 1940), however, has demonstrated the formation of such separating walls in species of *Carex* and *Scirpus*. According to Dnyansagar and Tiwari (1956) delimiting walls exist between the functioning and non-functioning nuclei of the pollen grain and the septa are also present between three non-functioning nuclei. The present investigation, therefore, has been undertaken with a view to learn the correct state of affairs in respect of these points in *Cyperus tegetum*. While studying these features the authors could gather detailed information on the development of the female gametophyte and consequently these observations are also included in the present communication.

Material and Methods

Cyperus tegetum Roxb. is a robust sedge growing abundantly in water-logged or marshy areas along the banks of the rivers and tanks throughout India. Flowers and fruits are available for about eight to nine months in a year from July to February.

The material was fixed in Formalin-acetic-alcohol at about 11 A.M. It was dehydrated and embedded in paraffin as per routine methods. Sections were cut at the thickness of 8–12 μ. Heidenhain's iron-alum-Hæmatoxylin was used as stain. The slides were destained in a saturated solution of picric acid and counterstained with fast green, especially to study the cell-walls between the nuclei enclosed in a common envelope of the pollen grain.

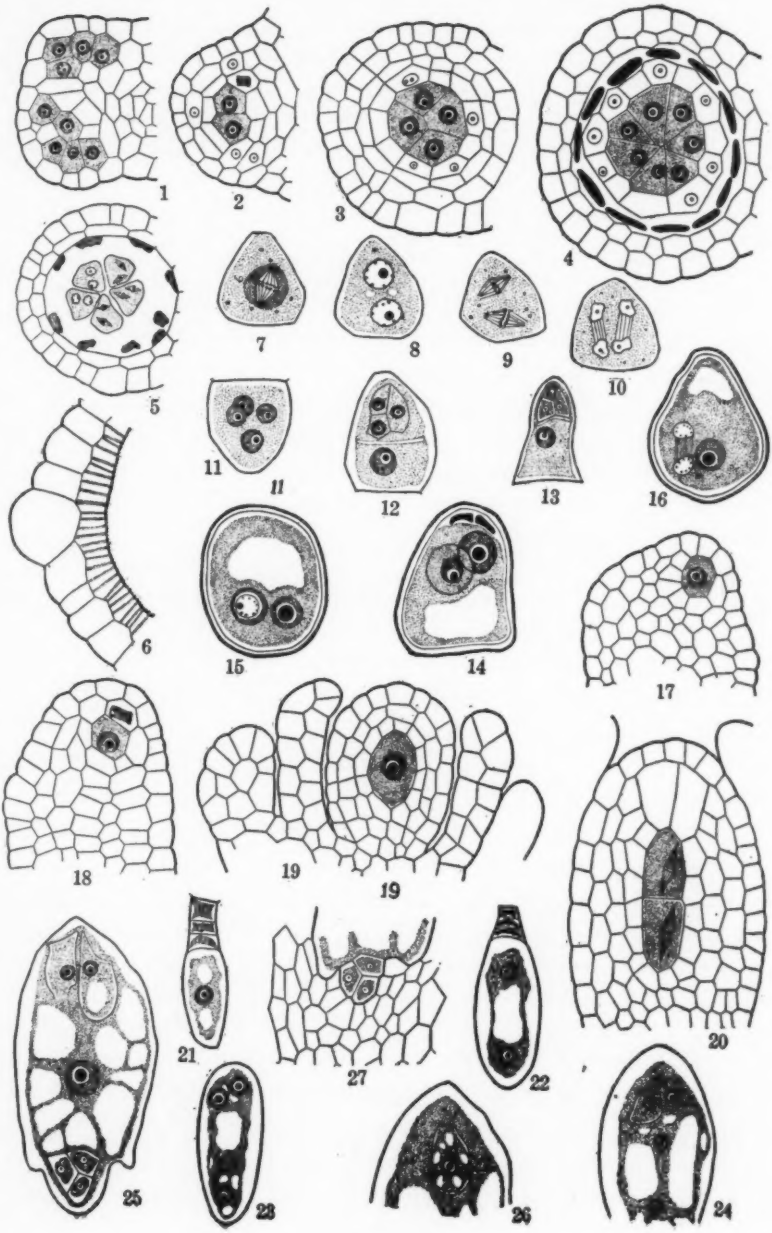
MICROSPOROGENESIS

The anther is bithecous. The hypodermal archesporium in the anther appears at four places and consists of 2 to 5 rows of cells extending over the whole length of anther (Fig. 1). The archesporium divides periclinally forming primary parietal and primary sporogenous layers. The primary parietal layer forms three layers, *viz.*, the tapetal layer, the early degenerating middle layer and the layer of fibrous endothecium (Figs. 2–4). Numerous globular markings appear on the inner tangential walls of the endothecial cells during later stages as in many other angiosperms (Fig. 6). The cells of the anther epidermis do not present uniform dimensions and at anthesis they are completely filled with yellow deposits.

Anther tapetum is of the secretory type and the cells finally become binucleate. Prior to degeneration the small yellow-staining globules (In slides stained with Hæmatoxylin) appear in large number on the inner tangential walls of the tapetal cells much before they appear on the fibrous endothecium. Such granules are now recorded in several angiosperms. For detailed information on this point reference may be made to the works of Ubisch (1927), Kosmath (1927), Kajale (1940), Puri (1941) and Singh (1950).

The primary sporogenous cells increase in size and divide once to multiply their number. The most unusual feature of the pollen mother cells is their shape as seen in transverse section. They appear triangular in outline with their apices meeting in the centre (Figs. 4 and 5). In longitudinal section, however, they look oblong and are arranged in two rows. During meiosis numerous irregularly distributed black staining bodies appear in pollen mother cells (Figs. 7, 9 and 10). In some cases these bodies disappear from the cytoplasm at a stage shown by Fig. 8; but they reappear during second meiosis. In others they continue to exist throughout the two meiotic divisions. The exact nature of these bodies is not known. Whatever be their nature or behaviour during earlier stages, no such bodies are seen in the young pollen grains.

According to Piech (1928) the pollen mother cells are elongated and the first meiotic spindle is parallel to its long axis. In *Cyperus tegetum*, on the



FIGS. 1–27

other hand, axes of the spindles during both the meiotic divisions are not oriented in any definite direction as in species of *Scirpus* (Piech, 1928). The four nuclei formed after the meiotic divisions lie free for some time in the cytoplasm and show no difference in their size at this stage (Fig. 11). The three nuclei now migrate to the tapering or proximal end of the pollen mother cell (Figs. 12 and 13). These are the non-functioning nuclei of the microspores. The functioning nucleus increases in size. All the non-functioning nuclei are of equal size and do not show any increase in their dimension. The septa are soon organized in between them and the four nuclei become separated from each other unlike in *Fimbristylis cericea* (Tanaka, 1939). Sometimes an additional septum may also be organized in between the functioning and three non-functioning nuclei (Fig. 12).

Thus as in other angiosperms, no regular tetrads are organized in the Cyperaceæ. These tetrads are termed as cryptotetrads by Erdtman (1952); though Selling (1947) prefers to call them pseudomonads as the pollen grains in the Cyperaceæ in general according to this author, are not homologous to those of other angiosperms.

The three non-functioning nuclei degenerate in course of time and their traces may be seen even beyond the two-celled stage of the pollen grains (Fig. 14). The uni-nucleate pollen grain gradually becomes vacuolate. Its nucleus divides to form a generative cell. The nucleus of the latter is slightly smaller than that of the other cell. In cross-section the generative cell looks circular as in *Scirpus lacustris* (Piech, 1928) (Fig. 15). It further divides and forms two male cells. The mature pollen grains in *Cyperus tegetum* are thus three-celled at anthesis (Fig. 16).

MEGASPORANGIUM

The family is characterized by a single unilocular pistil having a solitary basal ovule (Lawrence, 1951). The latter is bitegmic as in other species of *Carex*, *Cyperus*, *Heliocharis* and *Scirpus* (Schnarf, 1931). Only the inner integument forms the micropyle and the outer one up to the mature embryo-sac stage takes no part in its organization. The integuments during the early stages of development appear in acropetal succession from below the level of archesporium (Fig. 18).

MEGASPOROGENESIS AND EMBRYO-SAC

The hypodermal archesporium is one-celled (Fig. 17). It cuts off a parietal cell which generally divides periclinally before the megaspore mother cell forms the megaspores (Figs. 18 and 19). Sometimes, however, the

parietal cell divides anticlinally forming two cells which become elongated parallel to the long axis of the ovule as shown in Fig. 20. Later on, however, these cells divide in both directions to form a parietal tissue of few cells in thickness.

The megaspore mother cell forms dyad (Fig. 20). Both the cells are of equal size and divide transversely once again to form a linear row of four megaspores (Fig. 21). The functioning (chalazal) megaspore increases more in length than in breadth (Fig. 21). The nucleus after three mitotic divisions forms an eight-nucleate embryo-sac of the *Polygonum*-type (Maheshwari, 1950) as reported by Hicks (1929) in *Cyperus natalensis* and by Dnyansagar (1956) in species of *Fimbristylis*. According to Schnarf (1931) species of *Carex* also have the *Polygonum*-type of embryo-sac.

The egg is flask-shaped and much vacuolated (Fig. 26). The two synergids have usual structure and are hooked (Fig. 25).

The antipodals are three small cells and are housed in a small chalazal pouch on either side of which as seen in longitudinal section the part of the embryo-sac becomes dialated (Figs. 25 and 27).

The polar nuclei meet somewhere in the centre of the embryo-sac. After fusion the secondary nucleus shows considerable increase in size (Figs. 24 and 25).

Hypostase

An unusual feature, hitherto unrecorded in the Cyperaceæ, is the differentiation of the group of meristematic cells at the chalazal end of the embryo-sac surrounding the pouch and its chalazal part (Fig. 27). These cells are rich in cytoplasm and stand out more prominently than the remaining cells of the nucellus. This is homologous to the hypostase.

Summary

The paper deals with the development and structure of anther, pollen grain, ovule and male and female gametophyte of *Cyperus tegetum*.

The male archesporium consists of two to five rows of cells. It cuts off a parietal layer which divides further and forms three layers of the anther wall excluding the epidermis. The cells of the latter contain yellow deposits in them. The tapetal cells are uni-nucleate but later on become bi-nucleate and degenerate. The tapetum is of the secretory type. Globular markings on the inner tangential walls of the tapetum and fibrillar endothecium are noted.

Pollen mother cells in transverse section are triangular in shape with their apices meeting in the centre of the anther loculus. The divisions in them are simultaneous. Numerous black-staining bodies appear in the cytoplasm of the pollen mother cells during meiosis.

The three nuclei of the microspores are delimited by cell-walls and may or may not be separated by an additional wall from the functioning microspore nucleus. The three nuclei are cut off on the proximal side. Pollen grains are three-celled at anthesis.

Ovules are crassinucellate, bitegmic and anatropous. The micropyle is formed by the inner integument. The hypodermal archesporium is a single cell. Parietal cell is cut off. The megaspore mother cell forms a row of four megaspores. The chalazal megaspore develops into an embryo-sac of the *Polygonum*-type. The egg is flask-shaped with narrow neck. The synergids have a normal structure and are hooked. The antipodals are three cells present at the chalazal end in a pouch-like structure. The hypostase consisting of parenchymatous cells is developed below the chalazal end of the embryo-sac.

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Explanation of Text-Figures

Figs. 1–27. *Cyperus tegetum.* Figs. 1–4. T.S. of anther lobes showing various stages in tne development of anther wall. Note the radial arrangement of sporogenous cells in Fig. 4. Fig. 5. T.S. anther lobe showing two-layered anther wall, degenerating tapetal cells and pollen mother cells in meiosis II. Note that meiotic spindles are not orientated in any definite plane. Fig. 6. Part of anther wall showing fibrous endothecium with punctate markings. Figs. 7–10. Pollen mother cell showing meiotic divisions. Note the presence of darkly stained bodies in the cytoplasm of the pollen mother cells and the perinuclear zone surrounding the spindle. Fig. 11. Pollen mother cell showing four free microspore nuclei. Figs. 12 and 13. Pollen grain showing three microspores delimited by cell-wall. Note also the additional septum between the functioning and non-functioning nuclei in Fig. 12. Fig. 14. Two-celled pollen grain showing two out of the three degenerating non-functioning microspores. Fig. 15. The same as above showing generative cell in cross-section. Note that the nucleus is preparing for division. Fig. 16. Pollen grain showing tube nucleus and the dividing generative nucleus. Fig. 17. L.S. apex of nucellus showing single archesporium. Fig. 18. The same as above showing megaspore mother cell and anticlinal division in the parietal cell. Fig. 19. Same as above at an advanced stage. Fig. 20. L.S. apex of nucellus showing meiosis II in dyad cells. Note the enlargement of the parietal cells. Fig. 21. Uninucleate embryo-sac with three degenerating megaspores on the micropylar side. Figs. 22 and 23. Two- and four-nucleate embryo-sacs. Fig. 24. Micropylar part of embryo-sac showing two young synergids and two polar nuclei. Fig. 25. Embryo-sac showing two synergids, secondary nucleus and three antipodals in chalazal pouch. Fig. 26. Same as above showing egg. Fig. 27. Chalazal part of the embryo-sac showing three antipodals and hypostase. Fig. 5, × 350; Figs. 1–4, 6 and 17–27, × 600; Figs. 7–16, × 935.

PROBLEMS AND TECHNIQUES INVOLVED IN TRACE ELEMENT ESSENTIALITY STUDIES WITH FUNGI—A CRITICAL APPRAISAL

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Essentiality of an element for fungi was determined in earlier days by chemical analyses of the mycelium and spores produced. Certain elements such as carbon, nitrogen, phosphorus, sulphur, potassium, magnesium, etc., were invariably found in the ash of all samples analysed, though in varying amounts. More recent developments in spectroscopy enable the detection of a host of other mineral elements in ashed samples (Steinberg, 1937; Richards and Troutman, 1940). The mere presence, however, of an element in fungus cells does not necessarily mean that it is essential (Lilly and Barnett, 1951).

In nature and in the laboratory, fungi come in contact with both 'essential' and 'non-essential' elements. Unless proved conclusively, there is no reason to believe that fungi take up and store in their structure only elements which are needed for their growth and development. Furthermore, it seems equally possible that certain elements, indispensable for normal growth and metabolism, may not form any analytically detectable parts of the products formed. Many of the metallic elements occur, and are needed, in only minute traces. For these reasons, the problem of essentiality is better approached in another way, namely, by omitting the element in question from an otherwise adequate medium of known composition, and noting the response of the organism to additions of the 'test' element in separate cultures. If the element concerned is essential there should be a significant difference between the deficient and supplemented cultures (Foster, 1949). This is, indeed, the preferred method of testing essentiality of elements, particularly trace elements, which are needed only in minute quantities.

Only one element may be studied at a time, by limiting that alone in an otherwise 'complete' medium. In the absence of one or more of other elements that may be essential nutrients, the addition of a particular element,

which might also be essential, may not produce any significant response. The addition of Fe or Zn alone to $CaCO_3$-purified medium did not improve growth of *Aspergillus niger*, since both were essential and hence had to be incorporated together (Steinberg, 1919).

Since essentiality of an element to an organism is studied by limiting the amount of that element, it is ideal that the element in question be completely absent from the environment provided. This necessarily involves that the materials employed be pure. The diversity in kind and quantity of trace elements introduced as impurities in the experimental materials ordinarily used poses a very difficult problem in such critical work. Even 'chemically pure' substances are far from being 'biologically pure' and interfere seriously with trace element essentiality work. The luxuriant growth of organisms often obtained in media where no special precautions have been taken to remove trace metals is excellent testimony to this fact.

The situation is quite comparable to vitamin deficiency studies where the effect of these growth factors is often masked by contaminations from the ordinary culture materials used. The difficulties seem all the more accentuated in trace element work, since consistent trace element deficiencies show up only in rigorously purified synthetic media, whereas vitamin deficiencies have been demonstrated in media prepared from chemically pure substances. The possible reason seems to be simple in that the trace metals being free elements tend to occur as impurities in combination with other substances much more commonly than vitamins, which are themselves complex organic compounds.

Sources of Trace Element Contamination

The importance of trace elements as essential nutrients for fungi has been overlooked for a long time due to the non-recognition of these as impurities in the culture materials used, and of the wide difference between 'chemical purity' and 'biological purity' as we now know it. All the experimental materials employed, namely, chemicals, glassware, distilled water and inoculum, contribute to the nature and amount of these contaminations.

(i) *Chemicals.*—Although only refined, reagent grade chemicals are used in essentiality studies, they are by no means free from the biologically available trace elements. Steinberg's spectroscopic analysis of some of the chemically pure, reagent grade chemicals demonstrate the magnitude of the variety of heavy metals they contain as impurities (Steinberg, 1937).

The factors contributing to the nature and amounts of trace element impurities in chemicals and the inefficiency of the universal practice of recrystallization to prepare 'chemically pure' salts in rendering these substances 'biologically pure' are described in detail by Foster (1949). At least twenty elements tend to be co-precipitated with calcium (Hutner, 1946 *b*). Evidently, a medium prepared with such chemically pure substances, thus, need further purification for biological work.

Although spectroscopically pure chemicals have been used successfully in studies with Mo (Steinberg, 1937; Mulder, 1939), gallium (Steinberg, 1938) and calcium (Steinberg, 1948), it may not, perhaps, give a correct idea where certain other elements are concerned. That Zn was not detected in the elaborate spectroscopic analysis of the reagent chemicals (Steinberg, 1937) is interesting, since it is now known that this is one of the most commonly occurring heavy metal impurities (Foster, 1949). Moreover, the prohibitive cost of these high grade chemicals renders their use in routine work of this kind rather impracticable.

Recrystallization of individual salts has been considered unsatisfactory as a method of purification in this type of work (Roberg, 1928; Hewitt and Hallas, 1951). There are, however, a few reports where twice recrystallized salts have been found suitable for studies with certain elements—Mo (Mulder, 1948) and Ca (Basu, 1951).

(ii) *Distilled water.*—Unless repeatedly redistilled in high quality glass stills, distilled water may be a troublesome source of trace element contamination. Iron impurities in the distilled water used caused an abnormal reduction in citric acid production by *Aspergillus niger* (Perquin, 1938). Recent methods of ion adsorption using synthetic resins help to secure a quality of water much superior to ordinary distilled water. Double-distilled water was found to contain 5 μg. of solids per litre, including Pb, Cu and B (Cliquet *et al.*, 1913). Water redistilled in quartz is best. Nevertheless, water distilled twice in 'Pyrex' stills has been found to be generally satisfactory by many workers.

(iii) *Glassware.*—It will be a wasted effort to rigorously purify the medium and then contaminate it by the use of glassware which supply the elements. Many types of chemical glassware are sufficiently soluble, thus providing fungi with considerable amounts of these micronutrients. The absence of Zn from *A. niger* cultures had little effect when Jena glass flasks were used whereas, when 'Kavalier' or quartz vessels were substituted, it decreased the growth considerably (Javillier, 1914). Steinberg (1919) found a similar effect and noted that 'Pyrex' vessels were superior to 'Kavalier'

and equally suitable as quartz. It is now generally accepted that 'Pyrex' or 'Hysil' glassware are of sufficiently high quality for use in trace element work (Hewitt and Hallas, 1951). A recent report that American made 'Pyrex' vessels were somewhat superior to English made is interesting (Basu, 1951). Although quartzware is most preferable, it is too expensive and not generally available to the laboratory.

All glassware should be subjected to strong acid detergent cleaning, washed thoroughly and dried before use.

(iv) *Inoculum*.—The spores and mycelia of fungi are known to contain various mineral elements. They not only contain, but actually store trace elements (Foster, 1949). Hence, it has been held advisable to cultivate the inoculum on sub-maximal or even sub-optimal media in order to reduce the amount stored. Recently, however, precautions to remove trace metals from agar slant stock cultures of *Trichophyton persicolor* were reported to be of no advantage (Stockdale, 1953). It is far from desirable to introduce adhering medium into the deficient culture solutions along with the inoculum. As far as possible, dry spores should be used which may be gathered by a platinum wire. Steinberg (1935) noted an accentuation of Fe, Mn and Cu deficiencies when the spores were extracted with weak alkalies to reduce their mineral content.

From the foregoing it is evident that attempts to study the essentiality of trace elements call for a great deal of care and rigorously controlled conditions.

Purification Techniques

Steinberg's pioneering experiment which changed the whole concept of the biological functions of trace elements—from poisons to essential nutrients—clearly brought out the need for stringent purification of media employed in essentiality work. The trace element impurities of chemicals differ (Steinberg, 1937). Since each ingredient of the medium, the chemicals as well as distilled water, contains more, or less, of trace element impurities, the most practical approach is to purify the entire basal medium, instead of the several components separately.

Success in essentiality work with trace elements largely depends on the availability of efficient techniques to remove them from the media used. Various chemical methods of purification, falling roughly into two broad groups, namely, (i) those employing solid adsorbing agents like charcoal or $CaCO_3$ and (ii) those employing chelating agents which form insoluble complexes with heavy metals (Donald *et al.*, 1952 *a*), have been tried with

success by different workers. Steinberg (1919 to 1935) used his original $CaCO_3$ method to remove Fe, Zn, Cu and Mn. Activated carbon in alkaline solutions with a small amount of $(NH_4)_2S$ as a precipitant has been successfully used (Bortels, 1927; Roberg, 1928; 1931). Washed calcium phosphate (Sakamura, 1936) and precipitated aluminium hydroxide (Shu and Johnson, 1948) have been used, with controlled pH.

Special methods to remove particular elements have also been employed. Bertrand (1912) oxidized Mn to MnO_2 and removed it by adsorption on insoluble $MgNH_4PO_4$. Traces of Cu have been successfully removed by an electrolytic method (Wolff and Emmerie, 1930). Traces of Mo have been removed by co-precipitation with other sulphides by passing H_2S gas through acidified solutions (Nicholas, 1950; Hewitt and Hallas, 1951).

Trace elements have been removed efficiently by the use of chelating agents like oxine, dithizone and carbamate (Waring and Werkman, 1942; Zentmeyer, 1943, 1944; Nicholas, 1950; Hewitt and Hallas, 1951). Hydrogen-ion concentration exerts a profound influence on the complex-forming ability of these reagents and hence it is important to adjust the pH of the solutions to be purified to the required levels for the respective elements. Although leaving these reagents in the medium has been suggested, the inhibition in growth of organisms being due to immobilization of the essential ions (Albert *et al.*, 1947; Zentmeyer, 1944), it is desirable to remove the complexes after purification rather than leave them in the medium where the fungi are grown, many of which alter the pH to a great extent during their growth.

An interesting method of biological purification has been used as early as 1892 by Molisch, and since then by others (Roberg, 1928; Molliard, 1929; Waring and Werkman, 1942) to remove residual traces of Fe. *Aspergillus niger* was inoculated and allowed to grow in the impure solutions until its growth was limited by the exhaustion of Fe. The filtered solution then served as the basal medium. More recently, McLeod and Snell (1947) have used this method in studying the mineral nutrition of some lactic acid bacteria. 'In the final analysis, the biological test is the most critical criterion of purity' (Foster, 1949). Nevertheless, the biological method of purification does not seem advisable, considering the variety of metabolic products excreted by the growing organisms, which alter the composition of the medium much. The author's own attempts to eliminate the residual traces of heavy metals in a $CaCO_3$-purified medium, by growing *A. niger* for 48 hours, were not successful; indeed, the fungi grown in such a medium produced greater weight than in the chemically purified medium.

Donald *et al.* (1952 *a*) have reported results of a comparison of all the available chemical methods of purification. Thirty-eight methods—modifications and combinations—have been tried, using *A. niger* 'M' strain to test their efficiencies. They found the Al_2O_3 method (devised by them) or dithizone + oxine methods as most efficient to remove Fe and Zn, the Al_2O_3 method as the best to remove Cu and Mn and Nicholas' method of sulphide co-precipitation to remove Mo.

The diversity of the methods used to eliminate the different trace elements emphasizes the complexity of the problem associated with the preparation of media for such pure culture work. Media containing organic sources of nitrogen present special problems in purification (Steinberg, 1942; Hutner, 1946 *a*; English and Barnard, 1955). The lack of efficient available techniques may explain the limited number of elements proved to be essential for the organisms studied. A synthetic medium has been defined as 'one of known composition'. 'The important condition is that the compounds used be pure' (Lilly and Barnett, 1951). The brands of chemically pure substances readily available at the present day are far from being 'biologically' pure. If the latter were available, the preparation of basal media for essentiality work would have been much easier than at present.

Apart from preventing contaminations, as far as possible, from these major sources, careful manipulation of the many minute details in the experimental set-up, such as avoiding dust and indiscriminate use of glassware as far as possible, careful handling of chemicals and washing of glassware *et cetera*, has its own special value in such work.

Thus, obtaining a set of experimental conditions satisfactory enough to study the indispensability or otherwise of trace elements, particularly to micro-organisms, is no easy task. Growing the organism of interest in an environment completely free from the element under test is the ideal, but this is difficult to attain in practice. The experimental difficulties increase as the amount of element required becomes less (Lilly and Barnett, 1951). The situation is further complicated by the fact that different organisms have varying needs for these elements. The degree of purity to be attained depends to a great extent on the sensitivity of the organisms concerned.

Criterion of Purity and the Elegance of the Biological Test

'Analytical methods for the determination of the heavy metals (Fe, Zn, Cu and Mn) are, unfortunately, of insufficient sensitivity or precision to permit of determining the effectiveness of the method of purification' (Steinberg, 1935). The very sensitive chemical tests available at present

can detect considerably low levels of heavy metals, such as 0·1 mg. of Fe (*cf.* Foster, 1949), 0·01 mg. of total metals including Zn and Cu (Stout and Arnon, 1939) and 0·002 mg. of Mo (Piper and Beckwith, 1948) per litre. Nevertheless, when chemical methods fail to detect the metals in a culture solution, they may be present in sufficient quantity to support adequate growth of fungi. This is best demonstrated in the case of Mo; the fungus *Aspergillus niger* 'M' strain needs only 0·05 μg./50 ml. of this element for its normal growth and sporulation (Nicholas and Fielding, 1951), whereas the minimum amount detected by the dithiol reaction is only 0·1 μg./50 ml. Consequently, any amount smaller than this level, whether 0·05, 0·005 or 0·0005 μg., would remain unnoticed with this test, while growth and sporulation of the 'M' strain would show a great degree of variation from 0·0001 μg. level (thin, mucous hyphæ alone), through 0·005 μg. (thicker mat, but no sporulation), to 0·01 μg. (thicker undulating mat and sparse sporulation), and would attain the normal state of much wrinkled mat and complete, black spore cover at 0·05 μg. level, much lower than the minimum detectable by the chemical test. 'The astonishing sensitivity of this organism (*A. niger*) to infinitesimal quantities of certain of the trace elements in a rigorously purified basal medium is evidenced by a two-fold dry weight increase caused by one part per billion of Zn and by a large increase in growth with 1 part of manganese per 10 billion in the nutrient medium' (Foster, 1949).

In their ability to respond to very minute traces of nutrient substances, micro-organisms become of immense importance to science. Various substances like vitamins, amino acids and mineral elements in diverse materials are quantitatively evaluated using specific test organisms (*cf.* Lilly and Barnett, 1951). Their extreme sensitivity, combined with the ease with which they are handled for assay, as compared to the elaborate chemical tests involving various steps, special apparatus and numerous reagents, make them preferable to analytical methods. Specificity and stability of the organisms used are as essential as sensitivity for biological assays. Indeed, this accounts for the limited number of organisms chosen in practice, as against the theoretically unlimited possibility.

Most microbiological assays depend upon the proportional response of deficient test organisms to the substances for which they are deficient. This occurs only within a limited range of concentrations, *i.e.*, until the deficiency is made up. They are most useful to detect minute traces of the nutrients which other analytical methods fail to detect. A knowledge of the specific requirements of the test organism is, obviously, a necessary prerequisite before it could be used in specific tests.

Aspergillus niger 'M' Strain as Test Organism for Trace Elements and the Basis of the Bioassay Method

Mulder (1939) was the first to use this fungus to detect Cu and Mo in Dutch soils, and since then many workers have used his strain to assay Fe, Zn, Cu, Mn and Mo in various materials (Nicholas and Fielding, 1947, 1950, 1951; Nicholas, 1950; Hewitt and Hallas, 1951; Dole, 1952; Donald *et al.*, 1952 *a*, *b*). The principle involved is Liebig's 'Law of Minimum'. As a result of pure culture work, this organism has been shown to require, in addition to the generally recognized macronutrients, the micronutrients Fe, Zn, Cu, Mn, Mo, Ga, and possibly V and Sc, for normal growth and sporulation. The absence of any one of these from the medium results in characteristic deficiency symptoms. Furthermore, the increase in growth and spore intensity from deficient to normal state is directly proportional to increasing amounts of the element under test. Thus, it has been possible to prepare growth standards by adding known amounts of the 'test' nutrient to a series of culture solutions adequate in other respects. The standard series for the trace elements, according to Nicholas (1950) are: Fe, 0·1–20; Zn, 0·1–10; Mn, 0·01–10; Cu, 0·05–2·0 and Mo, 0·00005–0·02 μg./50 ml. of culture solution.

The 'M' strain has been found to be quite stable in its trace element requirements and hence its use as the test organism. Growth and the colour and intensity of sporulation are the criteria of assessment. The characteristic deficiency symptoms are:—

(1) Fe deficiency—markedly reduced growth and sporulation.

(2) Zn deficiency— do.

(3) Cu deficiency—decreased growth and no sporulation.

(4) Mn deficiency— do. The sterile mat much wrinkled and/or perforated.

(5) Mo deficiency—Much reduced growth and no sporulation.

Growth is not reduced in Cu- and Mn-deficient media to the same extent as in others. In fact, the use of dry weight yields for assessing Cu is not as sensitive as for Fe, Zn or Mo (Nicholas, 1950). Copper, however, has a specific effect on spore colour which changes gradually from white, through yellow, yellowish brown and shades of brown to the normal black.

Many recent workers who have used strains of *Aspergillus niger* (the more widely used being the 'M' strain) as biological test organisms have considered this method as superior to analytical methods (Mulder, 1939, 1948;

Gerretsen, 1948; Nicholas and Fielding, 1947, 1950, 1951; Nicholas, 1950, 1952; Hewitt and Hallas, 1951; Donald *et al.*, 1952 *a*, *b*; Dole, 1952). This fungus is used not only for evaluation of trace elements, but also macronutrient elements like K, Mg (Nicholas, 1950) and P (Manil *et al.*, 1956). Nicholas and Fielding (1951) suggested that the study of more strains and species of fungi and bacteria may reveal organisms which are, perhaps, more sensitive as biological test organisms than the ones used at present.

Results of the author's experiments with the 'M' strain of *A. niger* in evaluating the efficiency of techniques for study of trace element essentialities to fungi have already been published (Saraswathi-Devi, 1954). During that work, another strain of the same fungus was found to be less sensitive to traces of Mn, but much more so to those of Mo and Cu. In subsequent repetitions also, the new strain retained its extreme sensitivity to Mo and it was not possible to produce Mo deficiency symptoms in this strain with the method employed.

Under the standardized conditions, using the biological test, the essentiality of Fe and Zn for normal growth of nine species of *Fusarium* has been proved, but the indispensability of Cu could not be demonstrated for the *Fusarium* species studied (Saraswathi-Devi, 1958).

A Short Discussion of Points to be Remembered

Although the question of variation in nutritional requirements of the different organisms studied is an important consideration in comparing results obtained by various workers, the disparity in the experimental conditions provided, which, as we now know it, greatly influence these requirements has added much to the existing confusions and uncertainties in literature pertaining to trace element essentialities. Although there are a number of purification methods for the basal media available, the actual degrees to which these elements are removed vary considerably with every method. This is sufficiently exemplified by the recent comparison of all available purification methods made by the Australian workers, using the standard 'M' strain as the test organism (Donald *et al.*, 1952 *a*). Moreover, using the same technique, different workers have obtained different results. For instance, the well-known $CaCO_3$ method, considered the best by Steinberg (1934) for studies with Fe, Zn, Cu and Mn, has been found to be less efficient in the hands of recent workers (Brian and Hemming, 1950), and in fact, not much use is being made of this technique, of late (Steinberg, 1950). Furthermore, with the same strain and same techniques, results obtained by different workers have been varied. Nicholas and co-worker (1947 to 1951) could produce acute Mo deficiencies in growth of *A. niger* 'M' strain, using H_2S

co-precipitation method of purification, while Donald *et al.* (1952 *a*) found it difficult to obtain such results, employing the same method. The Mo deficiency demonstrated in this laboratory also was not so acute as reported by Nicholas (Saraswathi-Devi, 1954). Last, but not the least, is the variation in degrees of purity attained in repeated trials by the same worker, even while using the same materials, techniques and organisms. The response of the 'M' strain in different experiments varied in degrees, both in the case of Mo (Hewitt and Hallas, 1951) and Cu (Dole, 1952). The author's experience also has been the same. The foregoing instances indicate the complexity of the situations encountered while attempting an investigation on essentiality of trace elements. In view of these, the need for sufficiently sensitive tests to assess the efficiency of techniques is obvious, and the elegant bioassay method suggests itself. Moreover, the use of an organism as the test, grown under similar conditions as other species of interest, has its special value, since the many minute details in the experimental set-up, besides securing a purified basal medium, all contribute equally to success in essentiality studies.

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(*Vol. XLVIII, No.* 1, *Page* 38)

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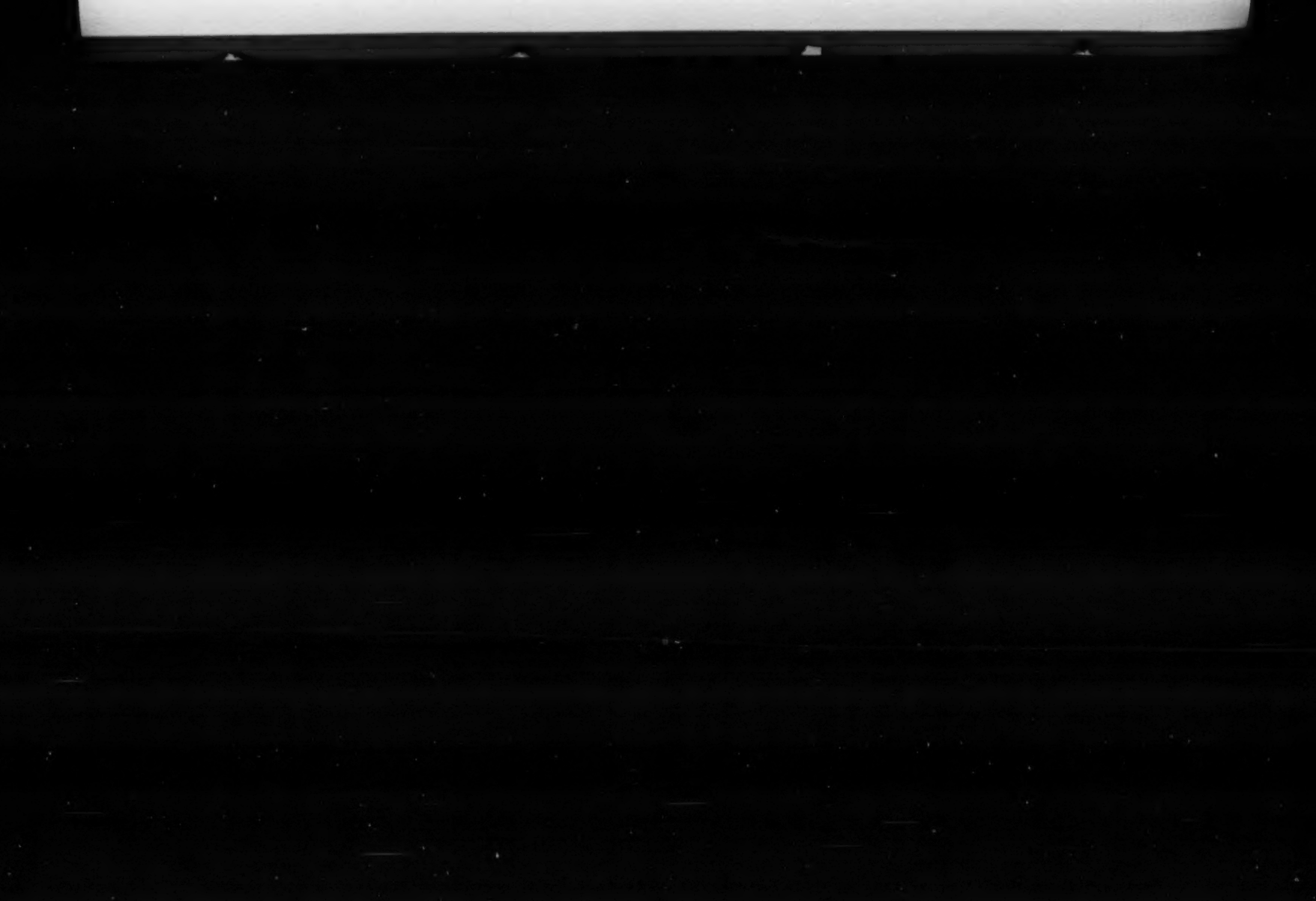

DRUG RESISTANCE IN YEAST (*SACCHAROMYCES CEREVISIÆ*)

Electrode Potentials of Primary and Secondary Colonies

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Received January 15, 1958

(Communicated by Professor S. N. Gundu Rao, F.A.SC.)

WHEN an electrode is put in a medium inoculated with a bacterial culture a potential is observed. Yudkin (1935) attributes this potential to be due to the effect produced by the bacteria and not due to the contact of electrode with the cells. The variation of this potential, therefore, shows the dynamic equilibrium between the activity of the enzymes and the constituents of the medium. In case of bacterial cultures, Winslow and Walker (1939), Potter (1911), Gillespie (1920) and Cannan, Cohen and Clark (1926) have shown that the metabolic activity of bacteria is intense during the lag phase when the oxidation-reduction potential falls, and there is a development of reducing conditions in the inoculated medium. Typical curves (Hewitt, 1931) relating to the electrode potentials of *Streptococci* and *C. diptheriæ* at various stages of development show a fall to a minimum value approximately equal to the duration of logarithmic phase. When death rate becomes appreciable the potential rises.

Kluyver and Hoogerheide (1934) made interesting observations on electrode potentials in cultures of different yeasts wherein differences in the conditions of fermentation and in different species of yeast were reflected in the potentials reached. Kakukawa (1938) found that the potential reached in living yeast suspensions was independent of indicator dyes. The phenomena of electrode potentials have been utilised by Hewitt (1950) to classify the bacterial cultures on the basis of peroxide formation.

The present authors (Sharma and Shukla, 1957) have shown that as a result of cultivating the yeast under increasing concentrations of Gentian Violet more resistant type strains develop. The trained strain when plated on drugged-agar medium gives two type of colonies, primary and secondary. The yeast cells arising out of these two types of colonies have been found to differ in regard to their fermentation activity (private communication) and lag phase (unpublished data). In the present paper study of the variation of oxidation-reduction potentials of the untrained strain (I.I.S.T. No. 13) in a drugged and non-drugged full rich synthetic medium, the primary culture

and the secondary culture in drugged medium, has been made in an attempt to differentiate the cultures on the basis of E_h/time curves.

MATERIALS AND METHODS

Isolation of two types of colonies from the trained strain

The solid medium used was the full rich White and Munn's (1951) medium solidified with 1·5 per cent. agar. The requisite amount of Gentian Violet was added to the medium to give a concentration of 8 mg./L. of the drug in the medium. The solid medium after sterilization was inoculated with different concentrations of cells. Two types of colonies appeared, large star-shaped termed by Hinshelwood (1956) as primary colonies and pinpoint colonies called the secondary colonies.

Measurement of electrode potential

The composition of the nutrient medium used was the same as reported earlier (Sharma and Shukla, 1957). Concentration of Gentian Violet in the drugged liquid medium was 8 mg./L. 10 c.c. of the synthetic medium were placed in each cell (*vide infra*) and the inoculum was $9{\cdot}9 \times 10^6$ cells for a twenty-hour old culture. The incubation temperature was $22 \pm 0{\cdot}1°$ C. Arrangement of the circuit for the measurement of the oxidation-reduction potential is shown in the sketch below.

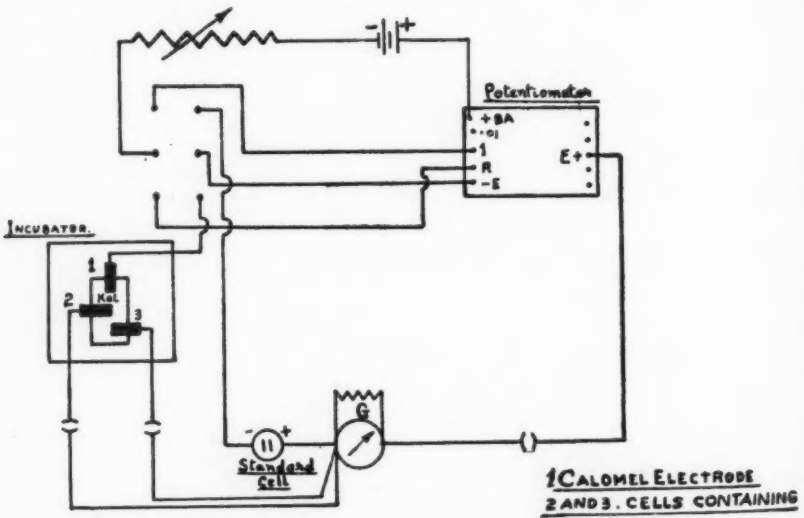

DIAGRAM OF CIRCUIT

The cells containing the cultures and the calomel electrode along with the saturated KCl reservoir were kept in an incubator at 22° C. and the leads connected as shown in the sketch.

Results

Figure 1 shows the electrode potential : time curve of the untrained parent strain in the drug-free medium and the drugged medium.

It will be seen that the E_h of the parent strain in the drug-free medium goes on falling steeply and reaches a negative value in about fifty-five hours. This denotes the highly reducing conditions obtained by the system. After this there is a rise of potential obtaining the voltages at which the medium was set up. The behaviour of the parent strain in the drugged medium

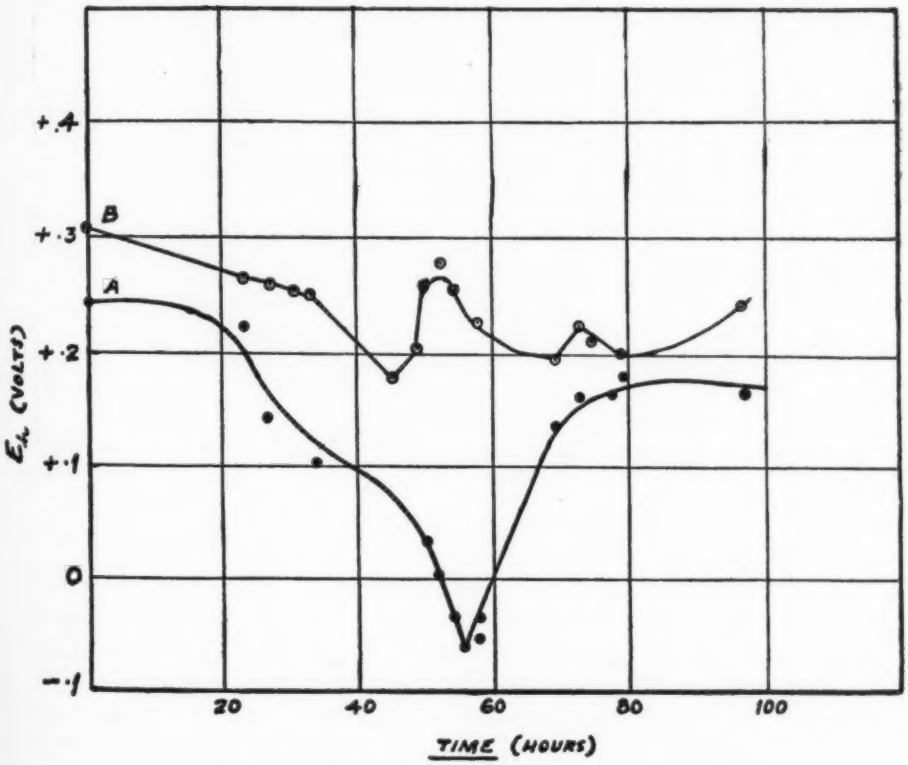

Fig. 1. Electrode potential: Time curves of the parent strain.
A. Drug-free medium. B. Drugged medium.

(Fig. 1, Curve B) shows that the E_h remains positive throughout and varies round a mean positive value after developing slightly reducing conditions. Figure 2 shows the electrode potential time curves of the primary and secondary colony cells in the drugged medium.

The reducing conditions obtained by the primary colony cells are similar to the untrained strain in the drug-free medium. The obtaining of the positive potential values is more time-consuming and the final values reached remain much lower than those initially at the setting time.

In case of the secondary colony E_h, time curve (Fig. 2, Curve B), it is seen that the oxidation-reduction potential varies round about a mean value with slight variation throughout the period of growth. The secondary strain behaves very nearly like the parent strain in the drugged medium.

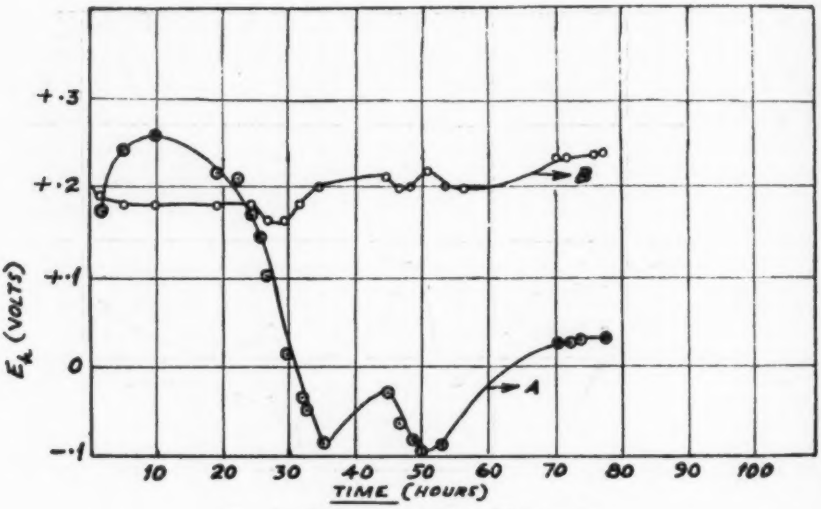

FIG. 2. Electrode potential: Time curves.

A. Primary colony in drugged medium. B. Secondary colony in drugged medium.

DISCUSSION

Hinshelwood *et al.* (1956) have reported that as a result of plating an untrained strain in a drugged medium, two types of colonies are obtained and that the secondary colonies appear to come from a mutant strain. This appears to be due to a sudden shock to the untrained cells by a comparatively high concentration of the drug. The training involves a gradual adaptation of a comparatively large number of cells which eventually become rate determining to increasing concentrations of the drug, so that in case of a trained

FIG. 1. A. Primary Colonies. B. Secondary Colonies.

strain, the primary colonies will only be formed by the adapted cells, while the secondary colonies will represent unadapted injured cells. This is borne out by Figs. 1 and 2 where the behaviour of primary colony cells in a drugged medium resembles closely to that of the untrained culture in a drug-free medium. The corresponding curve for the secondary colony cells approaches that for the untrained strain in the drugged medium. Furthermore, in case of the secondary colony it seems that the reducing enzyme systems get injured as a result of the drug action inasmuch as highly reducing conditions are never obtained throughout the growth. Further work on this aspect is in progress.

Summary

An attempt has been made to differentiate and classify primary and secondary colonies obtained from a trained strain on the basis of their E_h: time curves. It has been concluded that the primary colonies arise from adapted cells while the secondary colonies represent the unadapted injured cells.

Acknowledgements

The authors' thanks are due to Professor S. N. Gundu Rao, B.SC., A.H.B.T.I., F.A.SC., F.R.I.C., Director, National Sugar Institute, Kanpur, for his keen interest in this work and the Ministry of Food and Agriculture, Government of India, for the award of a Senior Research Training Scholarship to one of them (T. R. S.).

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NUCLEAR PHENOMENA OF SPORE-GERMINATION AND IN THE PRIMARY AND SECONDARY MYCELIA OF *AURICULARIA AURICULA-JUDÆ* (LINN.) SCHROET.

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Received October 8, 1957

(Communicated by G. P. Majumdar, F.A.SC.)

Introduction

Auricularia auricula-Judæ (Linn.) Schroet., the Jew's ear or Judas' ear, is well known and was described at least as far back as the end of the sixteenth century. Because of its widespread distribution and its ability to grow on a variety of wood, it has attracted the attention of the mycologists from time to time and its biology has been studied from various aspects. But so far the author is aware the available information regarding the nuclear phenomena of spore-germination and in the primary and secondary mycelia of the fungus is scanty. Brefeld (1888) germinated basidiospores in dilute culture solutions and obtained a luxuriant mycelium on which he did not find any clamp-connexion. Sappin-Trouffy (1896–97), who was the first to study nuclear details in the basidium of *A. auricula-Judæ*, incidentally mentioned that the fructification of the fungus was composed of hyphæ with binucleate cells, the nuclei being reduced to specks of chromatin. Green (1925), while reporting the occurrence of clamp-connexions in the fructifications of *Hirneola auricula-Judæ* stated that owing to the presence of deeply staining granules the nuclei and their relationship to clamp-connexions could be ascertained with certainty. An attempt has, therefore, been made to study the nuclear phenomena of spore-germination and in the primary and secondary mycelia of *A. auricula-Judæ* and a brief account has been presented in the following pages.

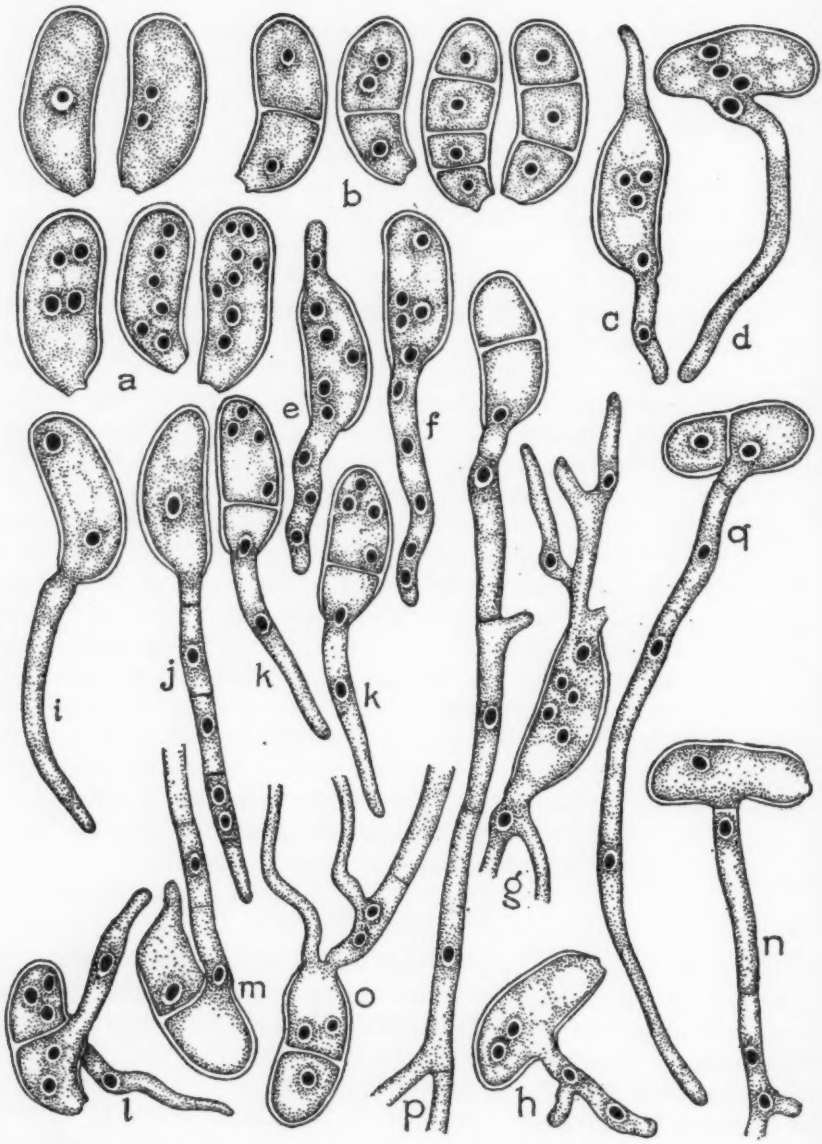
Methods

In this study, Kniep's (1913) agar film technique and that modified by Sass (1929) were mainly followed. In order to avoid undue external contamination special rectangular chambers as those recommended by Noble (1937) for hanging-drop-culture but large enough to hold agar films on slides were used. The composition of the medium was, however, slightly modified as follows: malt extract 2 per cent., gelatin 1·5 per cent., agar 1·5 per cent,

and distilled water 1,000 c.c. The constituents were dissolved in distilled water, filtered through a filter-paper in a steamer, tubed and sterilized in Koch's sterilizer at 100° C. When required, the medium was liquefied by heating in a water-bath, a few drops of liquid medium were placed on a warm sterile slide and spread as a thin film, taking care that the film did not reach the edges of the slide. As soon as the agar films had solidified they were inoculated with the mycelium, placed in an inverted position on rectangular chambers as stated before and kept inside sterile Petri-dishes, each containing a moist filter-paper from which a rectangular piece had been cut out at the centre for allowing light to be transmitted during examination. For obtaining germinating spores and primary mycelia, spores were directly dropped on the agar film from a piece of fresh sporophore. For early stages of spore-germination very thin films were used in order to obtain growing hyphæ more or less in the same plane. For growing and pairing primary mycelia of opposite sex to produce secondary mycelium, a comparatively thicker film was used so that the medium might not be used up quickly during mycelial growth. All these cultures were incubated at 28° C. in darkness and examined when necessary under sterile conditions. When the culture had reached the desired conditions, the slides were transferred to dry Petri-dishes and the films were allowed to dry down at the margin before killing and fixing were done. Several killing and fixing fluids were tried such as those of Flemming (weak), Bouin-Allen and Sass (1929). The visual results varied with the type of fixing fluid used but Flemming's fluid yielded the most satisfactory result. For early stages of germination, Sass' fluid was, however, found to be unsatisfactory and led to the bursting of the tips of young hyphæ. Various stains were tried but Flemming's triple and Heidenhain's iron-hæmatoxylin with Light green as counter-strain proved to be satisfactory. Of the two, Flemming's triple stain was particularly suitable for studying nuclei during early stages of spore-germination.

Observations

The nuclei of the spores have been stained readily and counted. It has been found that mature spores are mostly uninucleate although occasionally 2–8 nuclei have been observed (Text-Fig. 1 *a*). This may be interpreted as due to repeated division of the pre-existing nucleus of the spore. When germination starts this nucleus either divides into 2–4 daughter nuclei and transverse walls are laid down between them to form 3–4 uninucleate cells (Text-Fig. 1 *b*) or their number may increase by repeated division to form as many as 8 daughter nuclei within each spore (Text-Fig. 1 *a*). In the latter case when the germ-tube begins to develop at one or both ends of

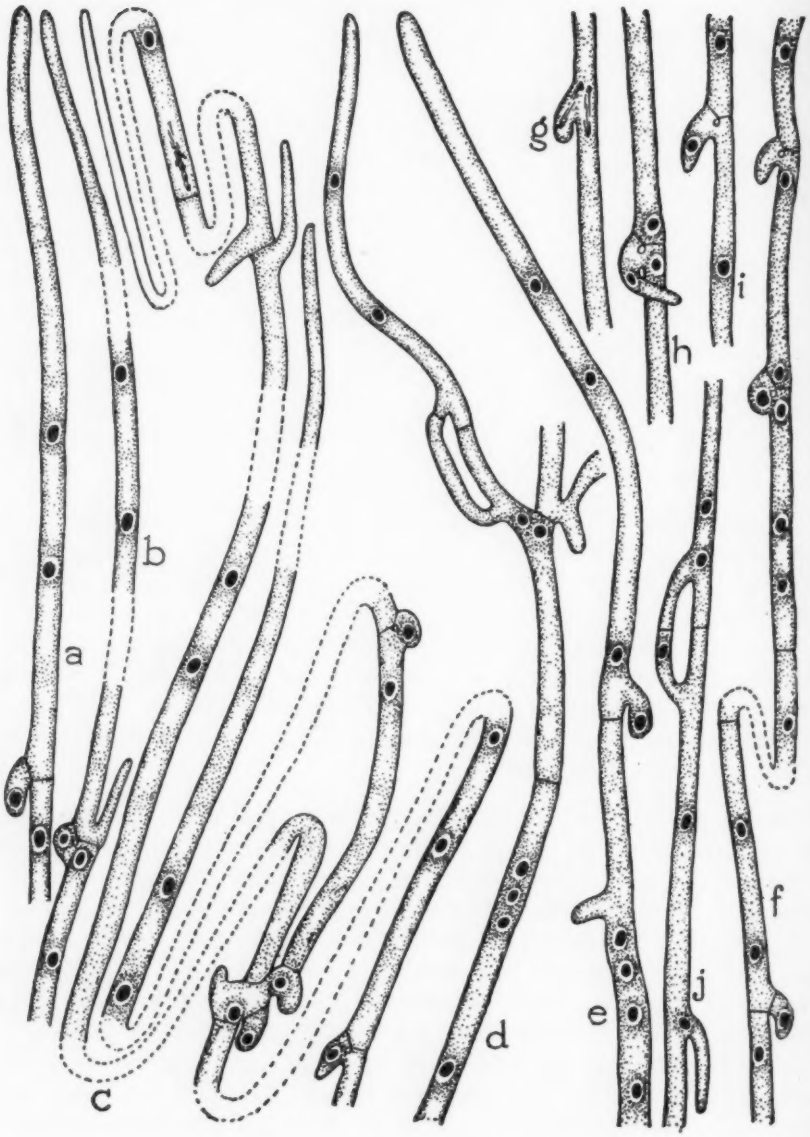


TEXT-FIG. 1. Nuclear phenomena of spore-germination and in the primary mycelium of *A. auricula-Judæ*. For explanation *vide* text (×1,400).

the spore, the nuclei migrate into the germ-tube and this coenocytic condition soon passes over into an uninucleate condition by more or less delayed formation of septa (Text-Fig. 1 *c* to *j*). The largest number of nuclei which has been observed in an unbranched tube is five (Text-Fig. 1 *f*). The end-cells are sometimes uninucleate but often each contains two nuclei (Text-Fig. 1 *j*). This is possibly due to the division of the pre-existing nucleus or delay in septum-formation. It has been observed that at the beginning these cross-walls are formed independent of the nuclear phenomena and they are rather difficult to demonstrate. When, on the other hand, the spore is divided into 2–4 uninucleate cells, the nuclei of one or more cells may remain undivided but the nuclei of those cells which give rise to germ-tubes always divide (Text-Fig. 1 *k* to *m*). and all or some of the daughter nuclei migrate into the developing germ-tubes (Text-Fig. 1 *o*) from which subsequently uninucleate hyphal cells are produced by the formation of transverse walls. The advanced primary or haploid mycelium consists of uninucleate cells and without clamp-connexions (Text-Fig. 1 *m*, *p*).

The structure of the fixed and stained nucleus has been described here. It practically occupies the entire width of the hypha (Text-Fig. 1*p*, *q*). Its nucleolus stains intensely with iron-hæmatoxylin and in a carefully destained preparation it is clearly visible. A clear space is discernible within the nucleus between the nucleolus and the nuclear membrane but no indication of any reticulum has ever been observed. The nature of a few deeply staining granules which frequently occur within the hyphæ (Green, 1925) could not, however, be determined with certainty. They are undoubtedly much smaller than the normal nuclei.

As a result of pairing between primary mycelia of opposite sex, the secondary mycelium bearing clamp-connexions has been obtained. In a properly fixed and stained preparation they are readily recognised but the actual place of origin of the secondary hyphæ is obscured due to the presence of a dense weft of highly anastomosed primary hyphæ at the line of contact. They can be easily traced back some distance into the culture rather than the actual place of origin. For this reason it has not been possible to study critically the details of the process of diplodization and conjugate division of the nuclei. However, the cells of the secondary mycelium are typically binucleate (Text-Fig. 2 *a*, *b*), although 3–4 nuclei with plane septa are not uncommon (Text-Fig. 2 *d*, *e*). This shows that the members of the conjugate nuclei can divide independently even when there is no question of clamp-connexion. The occurrence of clamp-connexion in the secondary mycelium is not, however, regular (Text-Fig. 2 *c*, *e*). The same hypha may



TEXT-FIG. 2. Nuclear phenomena in the secondary mycelium of *A. auricula-Judæ* and different types of clamp-connexions. For explanation *vide* text (×1,400).

bear plane septa as well as clamp-connexions. In the former case, this absence is possibly due to division of the nuclei of a conjugate pair independent of each other forming 3–4 nuclei but in the latter when simultaneous division of the two nuclei of a conjugate pair takes place, in which the two spindles are to be accommodated side by side in the narrow lumen of the hypha, a method for widening the hypha to facilitate division, is adopted resulting in the formation of clamp-connexion. Although no attempt has been made at present to study in detail the formation of clamp-connexion and the nuclear phenomena associated with it, yet from the structural point of view three types of clamp-connexions can be recognised as follows:

(1) In the first type the form agrees closely in salient points with the descriptions given by Bensaude (1918) and Kinep (1919–20). A short slender lateral pocket grows out from the side of the terminal hyphal cell, curves downwards, touches the main hypha (Text-Fig. 2 *b*, *e*, *f*, *g*) and ultimately fuses with it. Before or after fusion one cross-wall appears across each spindle of the dividing nuclei near the base of the pocket and across the hypha touching each other at an angle (Text-Fig. 2 *b*). In this type of clamp-connexion the terminal cell receives a pair of conjugate nuclei as soon as the division is complete but the sub-terminal cell and the lateral pocket (Text-Fig. 2 *a*) are both temporarily uninucleate. When the lateral pocket fuses with the sub-terminal cell the latter becomes binucleate.

(2) The second type agrees closely with the first type but the lateral pocket again slightly elongates, curves downwards and does not fuse with the main hypha. A gap is found between the point of contact of the two cross-walls and the elongated lateral pocket so that the nuclei can pass out easily through it from the sub-terminal cell below and re-enter the lateral pocket (Text-Fig. 2 *f*, *b*, *i*). This type of clamp-connexion is very common in *Auricularia auricula-Judæ* and although figured by Barnett (1937) has not yet been fully described.

(3) In the third type, which is uncommon, the lateral pocket instead of curving and fusing with the main hypha just below its place of origin, continues to grow like a small branch and after proceeding a little distance, curves and fuses with the main hypha so that when a clamp-connexion is formed a wide opening is seen between it and the main hypha (Text-Fig. 2 *j*).

It has been noticed that there is no constant time relationship between nuclear division, wall-formation and the fusion of the lateral pocket excepting in that the wall-formation which always takes place after nuclear division,

SUMMARY

1. In the present paper the nuclear phenomena of spore-germination and in the primary and secondary mycelia of *Auricularia auricula-Judæ* have been described. The information so far available was scanty.

2. The spores are at first mostly uninucleate. At the time of germination this nucleus either divides and 2–4 uninucleate cells are formed within the spore or the division is continued without wall-formation to form a multinucleate spore.

3. During germination the nuclei migrate into the germ-tubes and this cœnocytic condition soon passes over into uninucleate condition by more or less delayed formation of septa. These cross-walls are formed independent of the nuclear phenomena and they are rather difficult to demonstrate. Sometimes the nuclear division and wall-formation take place in succession so that from the beginning a primary mycelium of uninucleate cells is produced.

4. The cells of the secondary mycelium are typically binucleate although 3–4 nuclei with plane septa are not uncommon. The occurrence of clamp-connexions is not, however, regular. The same hypha may bear plane septa as well as clamp-connexions.

5. From the structural point of view three types of clamp-connexions have been described. It has been observed that there is no constant time relationship between nuclear division, wall-formation and fusion of the lateral pocket excepting in the wall-formation which always takes place after nuclear division.

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KARYOTYPES OF SOME VARIETIES OF BARLEY

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Introduction

Several varieties of barley (*Hordeum vulgare*) have been included among other cereals in the Mutation Research Programme, which is in progress in the Indian Agricultural Research Institute. In an attempt to detect any cytological changes induced by the treatment with different mutagens, the karyotypes of these varieties were critically studied. Bhaduri and Sharma (1949) and Sharma and Mukherji (1956) have studied the karyotypes of Indian varieties of barley. In our study, the varieties included by Sharma and Mukherji (1956) had been included. Our results, however, differed from those reported by them in many details and hence are presented in this paper.

Material and Methods

In the present study, the following six strains of barley evolved at this Institute were used:

1. Variety B–17
2. Variety B–42
3. Variety B–122
4. Variety B–134
5. Variety B–135
6. Variety B–137

Karyotype analyses were made from the study of somatic chromosomes from the root tip meristematic cells, fixed from freshly germinated seeds. Preparations were made as squashes after the technique of Bhaduri and Ghosh (1954). The procedure is described briefly below:

1. Pretreat healthy root tips with freshly prepared saturated solution of alpha-bromo naphthalene for 90 minutes.
2. Wash for 30 minutes in water to remove all traces of alpha-bromo-naphthalene.
3. Fix in freshly prepared mixture of 2% osmic acid, 1% chromic acid and 0·002 M 8-hydroxyquinoline (in the proportion of 1: 5: 1) for 2 hours.
4. Wash in water for 15 minutes and with 1% sulphuric acid for 15 minutes.
5. Mordant in 1% chromic acid for 1 hour.
6. Wash with water and hydrolyse in N HCl for 50 minutes at 60° C.
7. Wash with water and stain with leuco basic fuchsin.
8. Make a squash preparation of the brightly stained root tip portion in a drop of aceto-carmine and seal with wax.

The slides were examined when temporary and were made permanent by butyl alcohol method. Photomicrographs were taken by Leitz, Wetzlar 'Makam', IX, photomicrographic equipment, using Leitz, Wetzlar ocular periplan OK, 8 X and 90 X, Na. 1·3 oil immersion objective.

As a karyotype study requires careful observation, a good number of metaphase plates were studied and with the help of the camera lucida, accurate drawings were made. The length of the arms of the chromosomes were carefully measured and the idiograms drawn taking into consideration the size of the chromosome, position of the centromere and the number of constrictions. The measurements were made in five comparable plates and the average of these were taken into account.

Observations

The chromosomes are broadly divided into ten categories according to the length of the chromosomes and the position of the primary and secondary constrictions. They are:

A: Long chromosomes ($> 9\mu$) with sub-median centromere and a satellite in the shorter arm.

B: Long chromosomes with centromere sub-median and a satellite in the long arm.

C: Long chromosomes with median centromere without satellite.

D: Long chromosomes with sub-median centromere without satellite.

E: Medium chromosomes (8 to 9 μ) with sub-median centromere.

F: Medium chromosomes with median centromere.

G: Medium chromosomes with sub-median centromere and a satellite in the shorter arm.

H: Short chromosomes ($< 8\,\mu$) with median centromere.

I: Short chromosomes with sub-median centromere.

J: Short chromosomes with sub-median centromere and a satellite in the shorter arm.

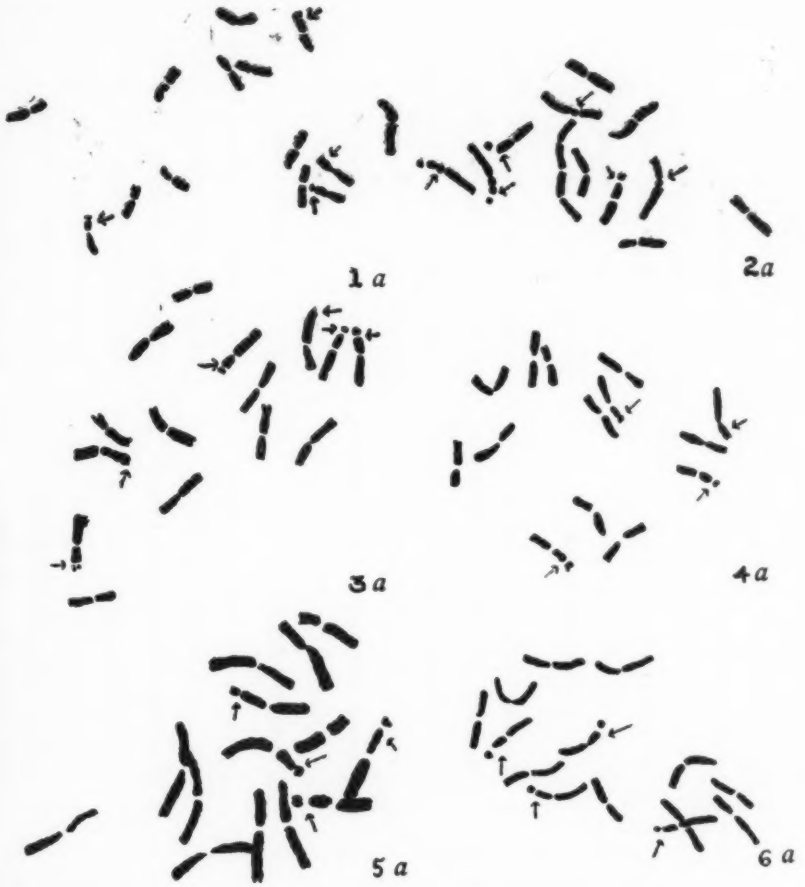
The chromosome types of each of the varieties are described below:

B–17 (*Figs.* 3, 3 *a*, 3 *b*).—(1) One pair of long chromosomes with sub-median centromere and a satellite in the short arm, (2) one pair of long chromosomes with sub-median centromere and a satellite in the longer arm, (3) one pair of long chromosomes with median centromere, (4) one pair of medium chromosomes with sub-median centromere, (5) one pair of medium chromosomes with sub-median centromere and a satellite in the shorter arm, (6) one pair of short chromosomes with median centromere and (7) one pair of short chromosomes with sub-median centromere. In this strain a pair of minute satellites (Fig. 3) were seen in the long arm of two chromosomes with sub-median centromeres. These satellites appeared as dots and were connected by very thin strands of chromosomes. It is interesting that a pair of chromosomes bearing an identical type of satellites were seen in *Agropyron triticeum* (Bhaduri and Natarajan, unpublished).

B–42 (*Figs.* 5, 5 *a*, and 5 *b*).—(1) Two pairs of long chromosomes with sub-median centromere and a satellite in the shorter arm, (2) two pairs long chromosomes with median centromere, (3) two pairs of long chromosomes with sub-median centromere and (4) one pair of medium chromosomes with sub-median centromere.

B–122 (*Figs.* 6, 6 *a*, 6 *b*).—(1) One pair of long chromosomes with median centromere, (2) two pairs of long chromosomes with sub-median centromere, (3) one pair of medium chromosomes with sub-median centromere, (4) two pairs of medium chromosomes with sub-median centromere

and a satellite in the shorter arm and (5) one pair of short chromosomes with sub-median centromere.

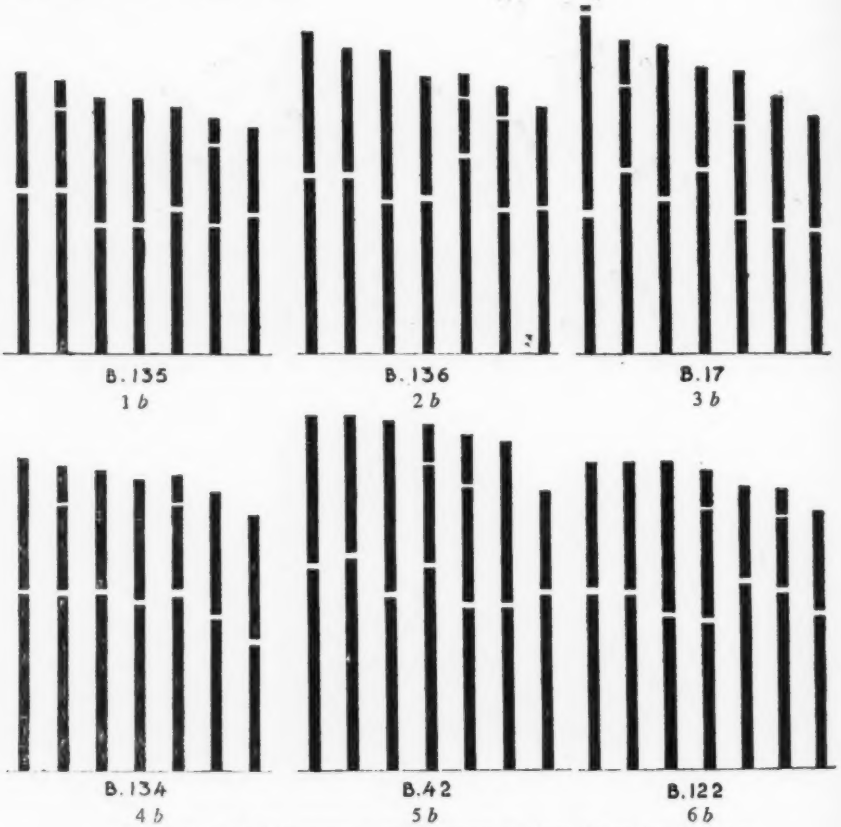


FIGS. 1–6. Drawings of the microphotographs in Figs. 1–6 of Plate XX.

B–134 (*Figs.* 4, 4 *a*, 4 *b*).—(1) One pair of long chromosomes with sub-median centromere, (2) three pairs medium chromosomes with sub-median centromere, (3) two pairs of medium chromosomes with sub-median centromere and a satellite in the shorter arm and (4) one pair of short chromosomes with median centromere.

B–135 (*Figs.* 1, 1 *a*, 1 *b*).—(1) One pair of medium chromosomes with sub-median centromere, (2) two pairs of short chromosomes with median

centromere, (3) two pairs of short chromosomes with sub-median centromere and (4) two pairs of short chromosomes with sub-median centromere and a satellite in the shorter arm.



Idiograms of the chromosome complements of these six strains of barley.

B–137 (*Figs.* 2, 2 *a*, 2 *b*).—(1) One pair of long chromosomes with median centromere, (2) two pairs of long chromosomes with sub-median centromere, (3) one pair of medium chromosomes with sub-median centromeres, (4) one pair of medium chromosome with sub-median centromere and a satellite in the shorter arm, (5) one pair of short chromosomes with sub-median centromere and (6) one pair of short chromosomes with sub-median centromere and a satellite in the shorter arm. In the strain B–137, out of the two pairs of satellited chromosomes, the longer pair showed a

double constricted nature. Sharma and Mukherji (1956) have reported in the same strain, besides the two pairs of satellited chromosomes, a third pair in which the secondary constriction occurs very close to the primary constriction. During the present study, a few cells were found, which showed such a type of configuration (Fig. 2).

Except in strain B–135, observations made by the present authors did not agree with those of Sharma and Mukherji (1956). The results are compared in Table I.

TABLE I

Variety	Number of chromosomes with					
	Satellites and secondary constrictions		Median centromere		Sub-median centromere	
	Sharma and Mukherji	Present work	Sharma and Mukherji	Present work	Sharma and Mukherji	Present work
B–17 ..	2	3	5	2	..	2
B–42 ..	2	2	4	2	1	3
B–122 ..	1	2	6	1	..	4
B–134 ..	2	2	4	1	1	4
B–135 ..	2	2	2	2	3	3
B–137 ..	3	2	3	1	1	4

DISCUSSION

Barley provides a good example of a crop plant which has undergone an extensive intra-specific differentiation without polyploidy or aneuploidy being one of the mechanisms involved in such a process. The innumerable number of barley varieties which are under cultivation in different parts of the world should therefore have come into existence by the genetic process of gene recombination by hydbriization, gene mutations and chromosome structural changes such as deletions, duplications, inversions and interchanges. The fact that many of the erectoid mutations isolated in barley by Gustafsson (1947) and his co-workers have also been characterised by specific translocations (Hagberg and Tjio, 1950) would suggest that chromosome interchanges

may be associated with the origin of economically desirable mutants. Burnham (1956) has described many interchanges of spontaneous origin in barley. Also, it is clear from the work of Ehrenberg *et al.* (1957) that even plants like barley which have been subjected to an intensive natural and human selection pressure over a long period of time may be encumbered with ancient and useless traits and that the easiest way to get rid of such traits is to produce a deficiency for them. No doubt deficiencies which lead, from the agricultural point of view, to beneficial phenotypic changes in the plant must also be occurring spontaneously. Probably such phenotypes are being either selected and cultivated directly or subjected to further recombination work.

The present study clearly shows that karyotype differences do exist among varieties of barley. The phenotypic distinctness of these varieties may to some extent be controlled and caused by spontaneous structural changes. Our results thus support the findings of Bhaduri and Sharma (1949) that chromosome structural differences provide a genetic basis for the wide varietal diversity found in barley. The karyotypes reported by us in this paper, however, differ in several respects from those described for the same varieties by Sharma and Mukherji (1956). This is apparently attributable to differences in the techniques adopted in the two studies. We have used the technique of Bhaduri and Ghosh (1954) and as the microphotographs would show the preparations are very satisfactory for the analysis of chromosome morphology. Only by using a critical and refined technique and by studying many cells in each strain, as was done during the present study, it will be possible to get a clear picture of the karyotype structure found among different varieties of a species. The task of varietal identification through the study of karyotype structure may not therefore be as simple as made out by Sharma and Mukherji (1956).

An observation of fundamental cytological interest relates to the identification of a pair of chromosomes which bear minute satellites in the strain B–17 (Fig. 3). Among the six barley varieties studied, this strain is unique in possessing three pairs of satellited chromosomes. Sharma and Mukherji (1956) reported only one pair of satellites in the strain B–122, but this variety really has two pairs (*cf.* Fig. 6). Such intervarietal differences in the number of satellite-bearing chromosomes have been reported in khapli wheat by Bhaduri and Ghosh (1954). Bhaduri and Natarajan (1956) have further shown that certain phenotypic characters tend to be correlated with the presence or absence of the extra pair of satellites. From an evolutionary point of view, the question arises whether the lower satellite number results

from an initially high number either by deletions or by a process similar to the 'amphiplastic' mechanism suggested by Navashin (1928) in *Crepis*. A second alternative could be that the high number of satellites is the result of translocations involving the satellite regions. Translocations involving chromosomes VI and VII (the satellite bearing chromosomes of barley) have been found in irradiation experiments (Hageberg, unpublished; reported by Ehrenberg *et al.*, 1957). Translocations affecting the satellite regions may also occur spontaneously and such translocations could lead to an increase in the number of satellited chromosomes. It is interesting that the additional pair of satellites in strain B–17 occurred in the long arm of Chromosome 1, while in the other chromosomes the satellites were found in the short arms. It would thus seem probable that this pair of satellites owes its origin to a translocation. On the other hand, the minuteness of the satellite heads and their resemblance to a pair of satellites found in *Agropyron triticeum* would indicate that this could be also a primitive feature which has disappeared in many present-day varieties. Further studies would be necessary to clarify this problem.

Summary

The karyotypes of six strains of barley are described and the results have been compared with those reported by Sharma and Mukherji (1956) for the same strains. The findings of the latter authors could not be confirmed in five strains, and the necessity to use critical cytological techniques and adequate number of cells for analysis in such studies is stressed. The role of chromosome structural differences in intra-specific differentiation is pointed out and it is shown that repatterning of the karyotype may sometimes result in genotypes of higher selection and survival value. The probable mode of origin of a pair of minute satellites present in addition to the normal two pairs in the variety B–17 is discussed.

Acknowledgements

We are thankful to Dr. B. P. Pal, Director and Dr. S. M. Sikka, Head of the Division of Botany for their interest in this study and encouragement. We are greatly indebted to Dr. M. S. Swaminathan, Cytogeneticist for many helpful criticisms, suggestions and for going through the manuscript.

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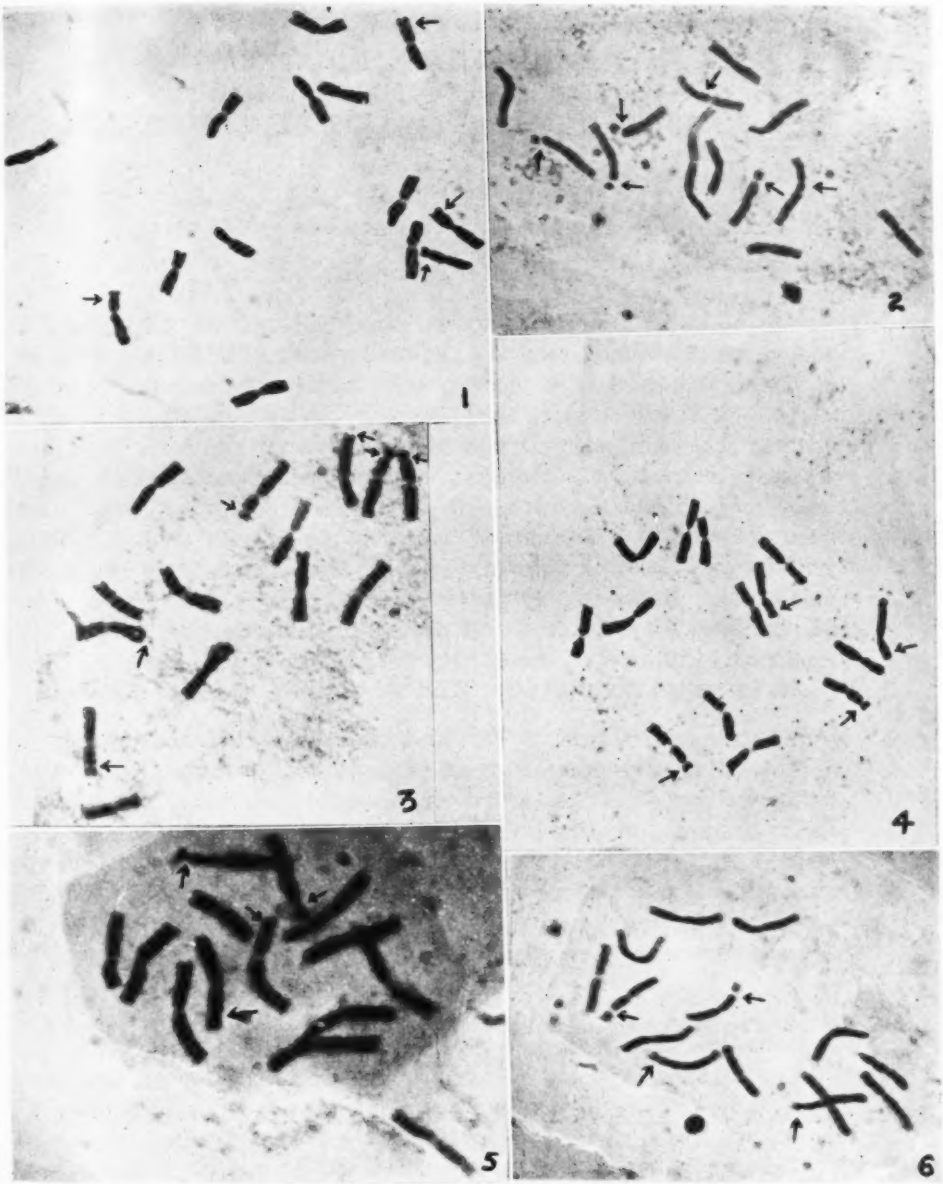

FIGS. 1–6. Somatic chromosomes of six barley varieties. The chromosomes with secondary constrictions and statellites are marked with arrow.
(1) B. 135; (2) B. 137; (3) B. 17; (4) B. 134; (5) B. 42; (6) B. 112.
(Magnifications of photographs: ×1050).

ABSENCE OF RESPONSE TO ADENOSINETRIPHOSPHATE IN A GLYCERINATED UNSTRIATED MUSCLE

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The enzymatic splitting of adenosinetriphosphate, providing the free energy of hydrolysis for immediate utilisation, has been considered the primary event in muscular contraction. Fleckenstein *et al.* (1954 *a*, *b*) found no evidence for ATP, ADP, or phosphocreatine changes after 1 to 2 second tetanus of the frog's rectus abdominis at 0° C. They reported of an increase in inorganic phosphate and of an unidentified fraction, suggesting the possibility of another source of energy-rich phosphate. Mommaerts (1954) found no evidence for the fission of either ATP or phosphocreatine during single twitches of turtle muscle; according to him "the possibility of a new 'Revolution in Muscle Physiology' is clearly discernible". Professor V. A. Engelhardt has spoken that this was not a revolution but only a change in cabinet. Dixon and Sacks (1958) using radio-active tracers, also found no evidence for ATP breakdown during contraction.

In attempting to study the effect of ATP on unstriated muscle of frog's stomach, it was noted that after the usual treatment with 50 per cent. glycerine and refrigeration, it does not respond to ATP with any contraction. Since this muscle shows the usual physiological responses found in muscle, the models of this muscle should respond to ATP like other muscle models, if this substance was the primary source of energy for muscular contraction. In the present research, therefore, a detailed investigation of the effect of ATP on the models prepared from this muscle has been undertaken.

Experimental

These experiments were performed on transverse pieces of the stomach muscle of the frog, *Rana tigrina*. After dissection, they were immersed in 50 per cent. glycerine and stored in a refrigerator at — 15 to — 20° C., for variable periods ranging from 24 hours to 6 months. Some muscles were stored at 0° C. The fluid was exchanged twice for fresh, precooled 50 per cent. glycerol, at daily intervals before storage. Before use the fibres were

placed in 15 per cent. glycerol at 0° C. for one hour; but 15 per cent glycerol causes marked lengthening of the muscle, and so is not suitable.

The effect of ATP was tested on loaded as well as unloaded pieces; the latter were used to test for active relaxation, if any. The experiments were performed at room temperature, 30° C.

RESULTS

As reported previously (Singh and Acharya, 1958), the effect of ions on frog's stomach muscle which has been refrigerated in 50 per cent. glycerine is different from that on striated muscle. Potassium chloride causes contraction of glycerine soaked striated muscle (Bozler, 1951). Similarly treated frog's stomach muscle from the cardiac end actively relaxes by about 60 per cent. in isotonic (0·112 *M*) potassium chloride. Calcium or magnesium (0·01 *M*) antagonises this action, so that the relaxation is reduced to 40 per cent.

In the present experiments, pieces of muscle were removed from the refrigerator and glycerine was removed by washing with potassium chloride solution, with or without magnesium. The treatment with potassium chloride solution continued till a constant length was reached in the freely floating condition. To test the effect of ATP on loaded muscles, they were suspended in a bath; to test for active relaxations, the preliminary solution was changed for that containing ATP with the muscle freely floating in a Petri-dish.

Effect of ATP on contraction.—The effect of the sodium salt of ATP in concentrations of 1·4, 1, 0·5, 0·25, 0·1, and 0·05 per cent. was tested. The muscles were treated beforehand with 0·112 *M* potassium chloride solution alone, or magnesium chloride in concentrations of 0·001 to 0·01 *M* was also added. In some experiments, calcium chloride in concentrations of 0·05 and 0·01 *M* was also included with or without magnesium in the potassium chloride solution. The ATP was dissolved in the preliminary solution with which the muscle was treated, but in concentration of 1·5 per cent. it was also used dissolved in distilled water. In over 50 experiments with loaded muscles, no contraction was ever observed. The muscles were stored in the refrigerator for 24–48 hours, 10 days, 1 month, 3 months and 6 months. In over 100 experiments with unloaded muscles, stored for 24–48 hours, 20 experiments with muscles stored for 10 days, 20 experiments with muscles for 1 month, 20 experiments with muscles stored for 3 months and 20 experiments with muscles stored for 6 months,

no contraction was ever observed. The addition of CP to ATP solutions made no difference.

Effect of ATP on active relaxation.—In the above 100 experiments with unloaded muscles, 12 pieces relaxed activity by about 10–40 per cent. (Table I). In muscles stored for longer time, no active relaxation has been even observed. As the positive results are few, these findings are of doubtful value. But it is also possible that the failure to get active relaxation by ATP in the great majority of experiments was due to preliminary active relaxation by potassium chloride, so that ATP was unable to cause any further relaxation.

TABLE I

Frog's stomach muscle. Active relaxation produced by ATP. Muscle previously soaked in 50 per cent. glycerine for 24–48 hours and then washed

Number of Muscle	Active relaxation per cent. of initial length	Solution
1	24	0·5 per cent. ATP in KCl
2	40	Do.
3	33	Do.
4	28	Do.
5	23	Do.
6	44	Do.
7	10	0·01 *M* $MgCl_2$ and 0·5 per cent. ATP in KCl
8	10	Do.
9	12	0·01 *M* $MgCl_2$ and 0·1 per cent. ATP in KCl
10	15	Do.
11	12	Do.
12	15	0·01 *M* $MgCl_2$ and 0·05 per cent. ATP in KCl

DISCUSSION

It appears that ATP does not cause contraction of glycerinated unstriated muscle from the stomach of the frog *Rana tigrina*. The living muscle behaves like any other muscle, so that these experiments throw doubt on the role of ATP as the primary source of energy in muscular contraction.

There is some possibility of active relaxation being produced by ATP. In this muscle, relaxation is attended with increase in oxygen consumption (Rao and Singh, 1940), and lactic acid production which is abolished by iodoacetic acid and cyanide along with active relaxation (Bharadwaj and Singh, 1951). Increased production of lactic acid during relaxation has been found by Mohme-Lundholm (1953, 1956); adrenaline, noradrenaline, isopropylnoradrenaline, and ephedrine have both a relaxing and lactic acid-forming effect. The degree of relaxation tends to run parallel with the increase in lactic acid concentration. Excess of calcium ions inhibit both the relaxing and the lactic acid-forming effect of adrenaline.

There is thus no doubt that there is breakdown of glycogen during relaxation of unstriated muscle, and as breakdown of glycogen is known to be preceded by fission of phosphocreatine and ATP, the production of active relaxation by ATP is theoretically possible; and so if the positive results in actual experimentation are few, nevertheless they are significant.

In unstriated muscle, ATP or some other substance might be acting on two different contractile mechanisms. There is evidence that unstriated muscle contains two contractile mechanisms, one of which relaxes actively and the other possively (Singh and Acharya, 1958).

The presence of two contractile mechanisms in unstriated muscle is shown by physiological experiments. Thus it shows two kinds of tone; one declines if the muscle is asphyxiated, but the other is unaffected by such a treatment; it may even increase (Singh, 1949). This shows that the former tone requires energy for its maintainence, but the latter is maintained without such expenditure of energy; it may last till the death of the muscle. The phasic response disappears if the muscle is asphyxiated, so that it would appear to be produced by the first contractile mechanism. This is shown by another experiment. In the dog's stomach muscle, it is possible to destroy tone by quick stretch, without affecting the phasic response, and the tone which is thus destroyed is that which is resistant to asphyxia (Singh and Singh, 1949). In the frog's stomach muscle, tone which is resistant to asphyxia, can be similarly destroyed, without affecting the phasic response (Singh and Singh, 1950), but if the tone, which is sensitive to asphyxia, is

destroyed by quick stretch, then the phasic response is also damaged (1957 *a, b*). This clearly indicates that there is one contractile mechanism which is responsible both for the phasic response and the tone which is diminished by asphyxia, and the other mechanism which is responsible for asphyxia-resistant tone. There is considerable other evidence for the existence of two systems in muscle (*see* Gelfan, 1958) for references.

Summary and Conclusions

1. Glycerinated frog's stomach muscle from *Rana tigrina* does not respond to ATP with contraction. As this muscle responds physiologically like other muscles, this throws doubt on the accepted role of ATP in muscular contraction.

2. In a few glycerinated muscles ATP has produced active relaxation. As the number of positive results is small, they are of doubtful value. But theoretically such a possibility exists as increased metabolism definitely occurs during relaxation.

I wish to thank the Indian Council of Medical Research for their generous grant for this research.

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MORPHOLOGY OF THE HEAD CAPSULE AND MOUTH PARTS OF *HELIOCOPRIS BUCEPHALUS* FABRE (COLEOPTERA, POLYPHAGA, SCARABÆOIDEA, SCARABÆIDÆ, COPRINÆ)

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I. Introduction

The majority of the Indian species of the family Scarabæidæ are included in the subfamily Coprinæ. Much of the knowledge of this subfamily is due to the great French entomological naturalist, J. H. Fabre (1857). As to the habits of the members of the group, Fabre's account has been supplemented by a description of certain Indian species by Major R. W. G.

Hingston (*cf.* Arrow, 1931). The members of this large subfamily have a very curious habit of dung rolling and are therefore commonly known as dung-rollers. They collect in dung, feed upon it, and make it into balls which they roll over the surface of the ground and take into the soil where it is also used by the young as food. The flat head is used as a shovel in these operations.

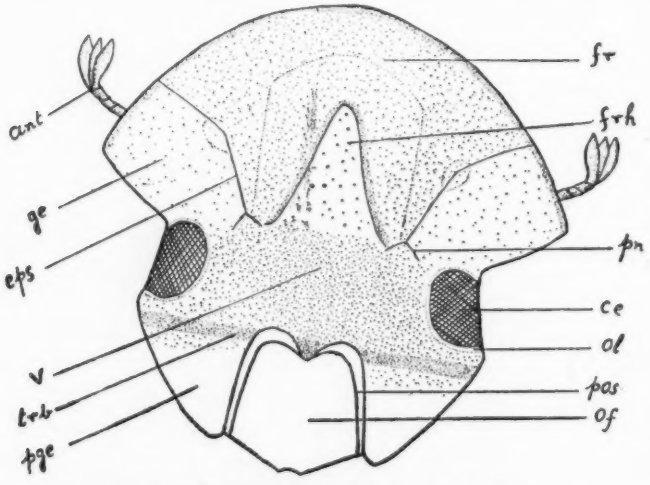
Heliocopris bucephalus Fabre is a giant of the family with a robust and globular body. Little information on the head capsule and mouth parts of an Indian species of the subfamily is available in literature. However, Smith (1892) worked out the mouth parts of an American species, *Copris carolina*. We have, therefore, ventured to describe the head capsule and mouth parts of *H. bucephalus* in detail.

II. Material and Method

The beetles were locally collected, generally at night, in the months of August and September, and were identified as *Heliocopris bucephalus* Fabre (Arrow, 1931). They were killed in potassium cyanide-bottle and preserved in 70% alcohol. The head capsule and mouth parts were made transparent by boiling in 10% solution of potassium hydroxide. The sclerites were stained in 2% solution of picro-indigo-carmine and the various structures were studied under a binocular dissecting microscope.

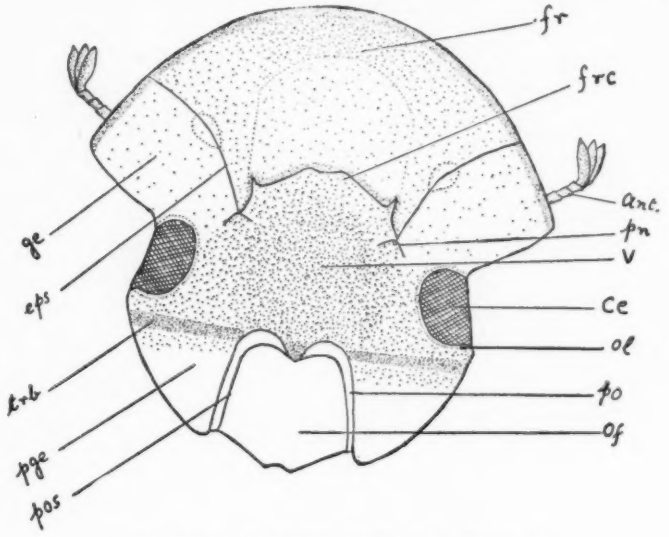
III. Head Capsule (Text-Figs. 1 *a* and *b*, 2 and 3)

The head capsule of *H. bucephalus* occupies a horizontal position in front of the thorax. It is a strongly chitinoid shield well suited to shovel

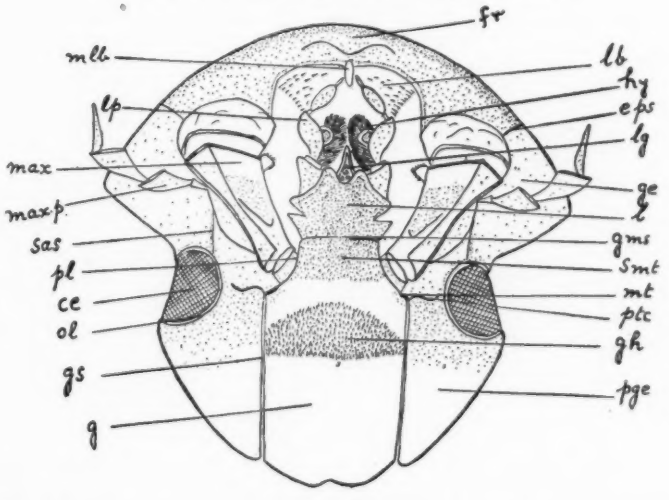


Text-fig. 1 *a*. Head capsule of male, dorsal view.

and roll the dung. The head capsule in *H. bucephalus* displays sexual dimorphism. On the middorsal surface in the male, there is a slender, curved and pointed horn, the frontal horn, at the fronto-vertex junction. At the

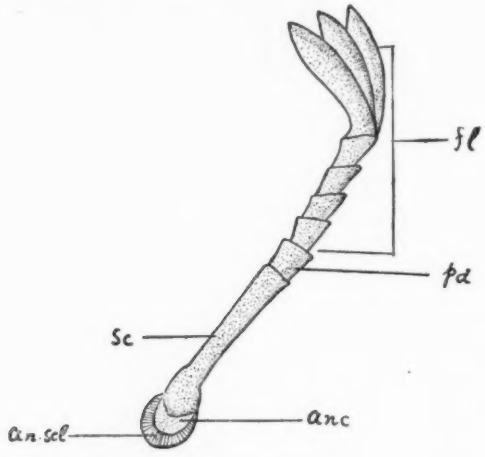


TEXT-FIG. 1 *b*. Head capsule of female, dorsal view.



TEXT-FIG. 2. Head capsule and mouth parts (antennæ and mandibles removed), ventral view.

corresponding position in the female there is a transverse carina, the frontal carina, sharply dentate at each end. The mouth is directed cephalad while the occipital foramen is at the opposite end.



TEXT-FIG. 3. Right antenna, dorsal view.

The epicranial stem is not visible in *H. bucephalus* and the epicranial arms are prominent being nearly straight sutures running obliquely across the head. Near the caudal end of each epicranial arm is a linear furrow, the pretentorina, which leads into the anterior tentorial arm. The area between the two epicranial sutures is the frons which forms the greater and middle portion of the shield. Arrow (1931) labelled this area as clypeus in *Catharsius molossus*. On the ventral side of the frons is a depression becoming deeper proximally, the presence of which is indicated on the dorsal side by a distinct impression of its outline. The clypeolabrum lies in this depression. Stickney (1923), however, does not mention in any member of the group Coleoptera the presence of such a cavity which is very well developed in *H. bucephalus*. Just in front of this depression is a short, distinct, sinuate ridge. The dorsal and ventral surfaces of the frons are granulate and papillate respectively, the papillæ being more compact on the ventral surface. All the mouth parts are situated on the ventral surface of the frons and are directed cephalad. On either side the shield presents a rectangular gena. The dorsal and ventral surfaces of the gena are granulate. Each gena has a compound eye at its laterocaudal end. The dorsal area between the compound eyes is the vertex which is closely granulate and slightly depressed.

Caudad to the vertex on either side is a triangular postgena separated from the vertex by a broad chitinoid transverse band.

The caudal end of the head capsule is formed by the occipital foramen surrounded by a distinct postocciput which is separated from the vertex and the postgena by a postoccipital suture. The postoccipital suture is inflected at the cephalic end. The postocciput bears two concavities on its inner margin for the articulation of the cervix and the anterior ends of the prothoracic muscles that move the head (Khatib, 1946). The occipital sutures are indistinguishable in *H. bucephalus* and thus the occiput is not marked off from the vertex and the postgenæ. Stickney (1923) regards the entire area extending from the occipital foramen to the vertex as the occiput. According to his terminology the area named as postocciput should be termed as occiput and the postoccipital suture as the occipital suture. Each compound eye is sickle-shaped and extends on the ventral side of the head forming thus a dorsal and a ventral faceted area on the respective side. Each eye is surrounded by a narrow sclerite, the oculata. No trace of any ocellus is found in *H. bucephalus* as is the case in other members of the group Coleoptera (Stickney, 1923).

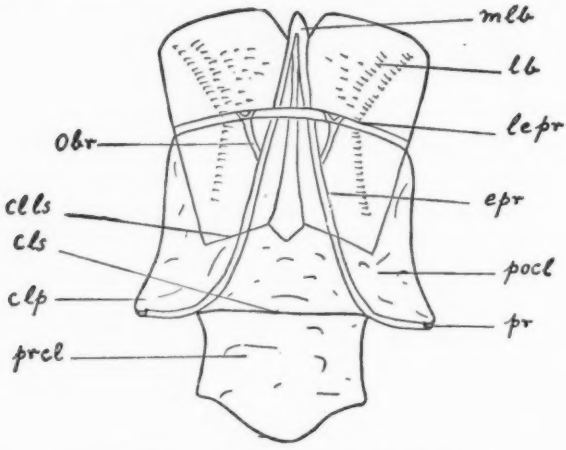
On the ventral side the broad area between the occipital foramen and the mentum, forming the middle portion of the head, is known as gula. It is separated from the remainder of the head capsule on either side by a suture, the gular suture. The gular sutures are subparallel. The cephalic margin of the gula is separated from the mentum by a well-developed gulomental suture. The midventral surface of the gula is setigerous. Each lateral side of the gula is surrounded by a large postgena. The dorsal and ventral surfaces of each postgena are granulate at the cephalic end and rugose elsewhere. The postgena at its cephalic end bears a cavity, the postcoila, for the articulation of the posterior articular condyle of the mandible of its side. On either side the cephalic end of the gula bears a crescentic socket, the paracoila, for the articulation of the two condyles of the cardo. The paracoilæ are chitinous.

On the ventral surface the head bears an antenna cephalad to each compound eye. Each antenna is lodged in a socket, the antacava, provided with a distinct antennal sclerite or antennarium with which the antenna movaby articulates by an arthrodial membranous ring, the antacorium. The antacavæ present an impression on the dorsal surface of the head. From the lower end of each antacava a straight suture, the subantennal suture, runs vertically downwards to end near the upper margin of the oculata. The position of the antenna is very interesting in *H. bucephalus*. It is situated

neither on the gena nor on the frons, but on the epicranial suture. While describing in general the head capsule in Coleoptera, Stickney (1923) does not mention such a position of the antennæ in any member of the group. Each antenna is lamellate (Essig, 1942) and consists of nine segments. The basal segment, the scape, is the longest of all, the second segment is the pedicel while the rest seven segments form the flagellum. The last three segments of the flagellum are leaf-like and folded together loosely.

IV. Mouth Parts

(*a*) *Clypeolabrum* (Text-Figs. 4 *a* and *b*).—Generally the cephalic margin of the frons is articulated with the caudal margin of the clypeus. The fusion of the frons with the preclypeus is sometimes indicated by an epistomial suture (frontoclypeal suture). Walker (1931), while describing the clypeus and the labium of primitive insects, states that the existence of the clypeus as

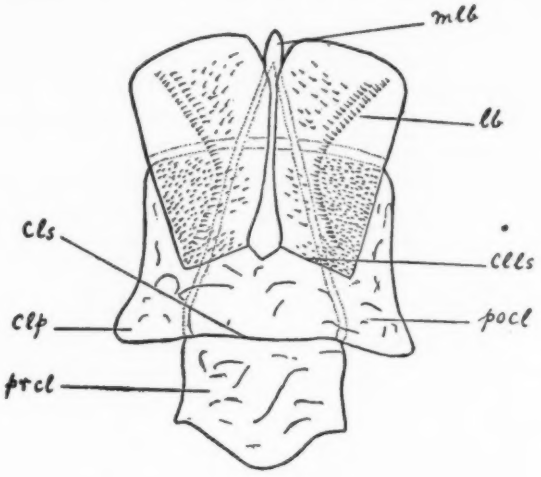


Text-Fig. 4 *a*. Clypeolabrum, dorsal view.

a distinct region of the head capsule is due to the epistomial suture. In *H. bucephalus* as in other Coleoptera, there is no trace of an epistomial suture. An interesting feature to be noted is the presence of the clypeolabrum in a depression on the ventral side of the frons, and as the frons and clypeus are not in the same level the question of an epistomial suture does not arise. The caudal margin of the clypeus is thus directly articulated with the cephalic margin of the hypopharynx.

The thin and membranous clypeus is composed of a single wall divided into two regions, the pre- and the post-clypeus, marked off from each other

by a faint transverse suture, the clypeal suture. The preclypeus is short and almost quadrangular broad region articulated with the hypopharynx. The postclypeus forms the larger part of the clypeus. On the ventral side the



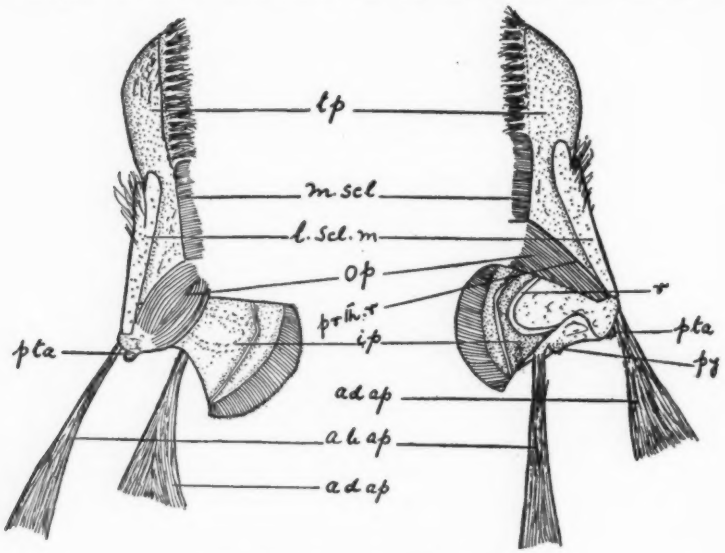
TEXT-FIG. 4 *b*. Clypeolabrum, ventral view.

lateral parts of the postclypeus are separated from the middle part by means of two longitudinal ridges running from the mesolabrum to the respective caudolateral end. The caudolateral part of the postclypeus on either side is projected into a lobe-like structure, the clypealia. Stickney (1923) mentions that in Coleoptera the clypealia is often separated from the postclypeus proper by a distinct furrow or suture. *H. bucephalus* bears a clypealia on either side which is not distinguishable from the postclypeus proper by means of a furrow or a suture except by its lobe-like projection.

The labrum is partly chitinous and is composed of two walls. It is divided into two parts which look like two leaves of an open book separated from each other by means of two narrow ridges with a gap between them which becomes wide distally. Each part is separated from the clypeus by an obliquely running clypeolabral suture. Between the two parts of the labrum on the cephalic end is situated a small membranous oval lobe, the mesolabrum. On the dorsal and ventral surface of the labrum are present many thickly-set, pointed, long and short bristles some of which are arranged in linear rows. The labrum bears many long and soft hair at its anterolateral margins too, while on the posterolateral dorsal surface it is beset with numerous pointed short hair directed inwards.

The presence of some prominent ridges on the ventral surface of the clypeolabrum (clypeal and labral epipharnyx) is to be noted. Just in the middle of the labrum is a broad transverse ridge, the labro-epipharyngeal ridge. From the middle of the mesolabrum run two longitudinal ridges, the epipharyngeal ridges, which become wide distally. The caudal end of each ridge is produced into a small cavity, the precoila, into which fits the anterior articular condyle of the respective mandible. The two longitudinal ridges are connected with the transverse ridge by means of another two short oblique ridges which bifurcate at their cephalic ends.

(*b*) *Mandibles* (Text-Figs. 5 and 6).—The mandibles are situated in the lateral margins of the preoral cavity and represent the most efficient organs

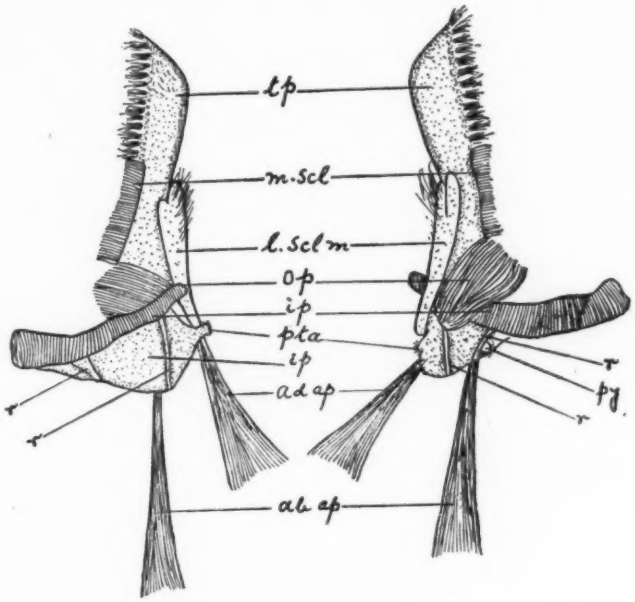


TEXT-FIGS. 5 *a* and *b*. Right mandible.
(*a*) Dorsal view.
(*b*) Ventral view.

for grinding. Each mandible has two articulatory processes at its base. The outer articular condyle, the postartis, fits into the postcoila at the cephalic margin of the postgena just caudolateral to the paracoila. The anterior articular condyle, the antartis, fits into the precoila formed by the caudal end of the longitudinal ridge on the ventral surface of the clypeolabrum.

The terminal part of each mandible is not designed for biting as is evident from its thin membranous structure with more or less rounded cephalic mar-

gin. It bears numerous long, thin, branched hair arising mesially from a distinct rectangular basal sclerite. Lateral to the basal sclerite is another chitinous semicircular sclerite beset with small hair on its cephalolateral side.



TEXT-FIGS. 6 *a* and *b*. Left Mandible.

(*a*) Dorsal view.

(*b*) Ventral view.

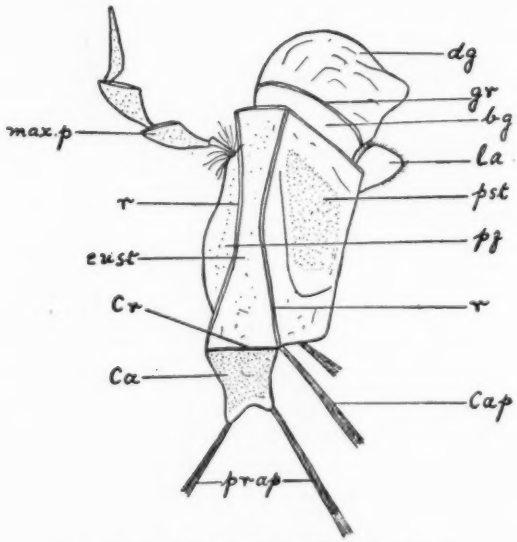
The basal part of each mandible consists of a large, hard and chitinous prostheca separated from the upper part by a well-developed prosthecal ridge on the ventral side. The prostheca is distinguishable into two parts—the outer and the inner. The outer thin and membranous part bears numerous long horizontal ridges on its dorsal surface. The inner part is in the form of a long, transverse comb-like structure provided with numerous short longitudinal ridges. Each comb is supported by two short ridges. These ridges remain separate in the left mandible while in the right mandible they unite at their cephalic ends to form a single semicircular ridge. The lower margin of this part of mandible is finely fringed with a single row of short hair. The inner part of the right mandible is nearly semicircular and is directed downwards while that of the left mandible is transversely elongated and directed upwards. The various ridges on the prostheca of one mandible work against

the corresponding ridges on the prostheca of the other; and thus the two mandibles constitute a most efficient grinding organ.

Between the terminal part and the prostheca on the mesial side is a rectangular mesial sclerite beset with numerous long and soft hair directed inwards. Just opposite the mesial sclerite on the lateral side of each mandible is another long and chitinous sclerite, the lateral sclerite. Its cephalic end is bifurcated and the caudal end terminates near the postartis. The dorsal and ventral surfaces of the lateral sclerites are coarsely granulate and the cephalolateral margin bears long hair.

At its base each mandible possesses two apodemes—an inner adductor and an outer abductor apodeme for the insertion of the adductor and abductor muscles respectively.

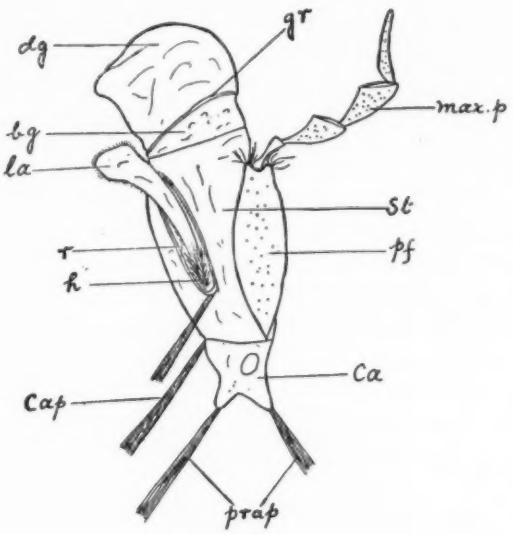
(*c*) *Maxillæ* (Text-Figs. 7 *a* and *b*).—The maxillæ are situated above the mandibles on either side of the mesially placed labium. Each maxilla articulates with the head capsule by means of the two condyles of the cardo which fit into a cavity, the paracoila. The cardo is made up of a dorsal



TEXT-FIG. 7 *a*. Right maxilla, dorsal view.

closely granulate and a smooth ventral wall. The space between the dorsal and the ventral walls of the cardo communicates to the exterior by means of an oval aperture in the ventral wall. The cardo shows no division into basi- and disti-cardo. The inner articular condyle of the cardo bears a long pro-

motor apodeme and the outer bears a short one. The stipes forms the main portion of the body of the maxilla and is divided into eustipes and parastipes by a longitudinal ridge on its dorsal side (Crampton, 1924). The eustipes and parastipes are not distinguishable on the ventral side. The stipes is separated from the cardo by a transverse cardostipital ridge, from the inner end of which originates a long cardostipital apodeme. The parastipes is a broad rectangular part of the stipes and bears a lacinia at its cephalic end. A distinct inverted walking-stick-shaped groove is present on the dorsal surface of the parastipes which is closely granulate in its middle. The eustipes is narrow and long and bears a palpifer at its midlateral side. In the ventral view the palpifer looks like a distinct laminate sclerite and on the dorsal side



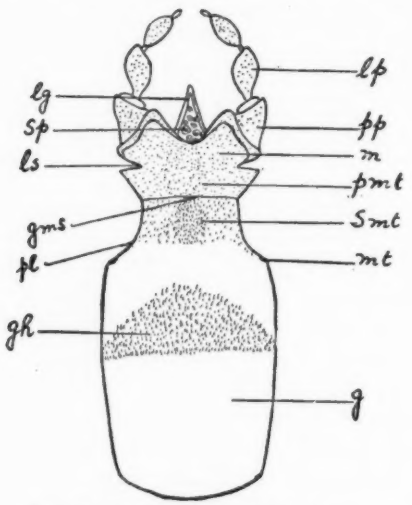
TEXT-FIG. 7 *b*. Right maxilla, ventral view.

it is separated from the eustipes by a longitudinal ridge running throughout its length. The dorsal and the ventral surfaces of the palpifer are granulate. From the cephalic end of the palpifer is given out a four-segmented maxillary palpus. The first segment of the maxillary palpus is short and narrow, its caudal end fitting into the cephalic concavity of the palpifer through an arthrodial membrane. The second and the third segments are long and cup-shaped while the fourth segment is also long but cylindrical. The last three segments are granulate on the dorsal and ventral surfaces. The lacinia borne on the ventral side of the stipes is a long membranous structure clavate at the

cephalic end and carrying short soft hair on its margins. The stem of the lacinia is visible only in the ventral view. Its mesial margin is pubescent. Throughout its length the stem is surrounded on either side by a longitudinal ridge borne on the stipes. The two ridges meet below the distal end of the lacinia. From the caudal end of this ridge runs downwards a long apodeme for insertion of muscles which bring about the movement of the lacinia. The caudal end of the lacinia is beset with numerous long black stiff hair pointing cephalad. The cephalic end of the stipes gives out galea which is divided into two parts by a transverse ridge. The lower, smaller and chitinous part is the subgalea, and the upper large and lobe-like membranous part is the distigalea. The distigalea is provided with very short and soft hair on its cephalic and lateral margins. The galea is separated from the stipes by a well-developed ridge.

The maxillæ are devoid of teeth and are incapable of biting.

(*d*) *Labium* (Text-Figs. 2 and 8).—In *H. bucephalus* the labium is reduced because of the greater development of the gula. The main body of the labium is formed by a mentum and a prementum which are marked off from each other by a labial suture. The labial suture is indicated by the presence of its lateral portions in the form of well-defined invaginations while the mesial portion of this suture is indistinct. The mentum is attached to the gula at its cephalic margin and is separated from it by a well-developed gulomental suture. The mentum and prementum are beset with numerous short and



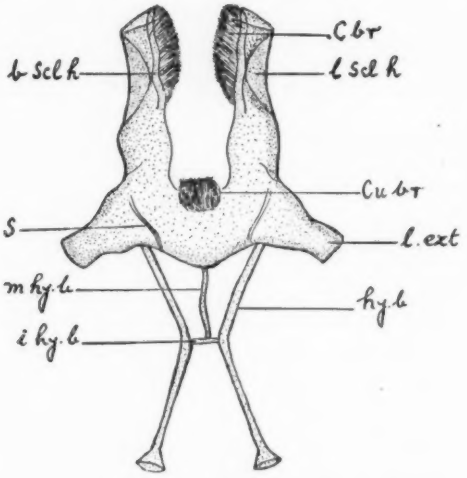
TEXT-FIG. 8. Labium, dorsal view.

pointed hair on the dorsal and the ventral surfaces. A small cephalic area of the gula on the dorsal side just below the gulomental suture also bears numerous short and pointed hair. This area may be an indication of the submentum which is otherwise not marked in the labium. This view is further supported by the position of the metatentorinæ situated at the caudal ends of the paracoilæ in the form of invaginations (Snodgrass, 1935). According to Crampton (1928) the region between the mentum and the gula is the submentum. Externally, the boundary of the submentum may be more or less arbitrarily demarked by drawing an imaginary line from the basal articulation of the cardo of the one side to the basal articulation of the cardo of the other side. Crampton further states that it is less satisfactory to attempt to use the gular pits (metatentorinæ invaginations at the caudal ends of the paracoilæ) or a line drawn from one gular pit across the other as landmarks indicating the posterior limit of the submentum because of the shifting of gular pits in an irregular fashion. As the basal articulations of the cardines do not seem to be subject to such disconcerting shiftings they account for a most satisfactory determination of the posterior limit of the submental region. But in *H. bucephalus* the basal articulations of the cardines and the gular pits are at the same position, thus both the views would apply for determining the posterior boundary of the submental region. There is nothing to differentiate between the gula and the submentum, as there is no suture between them, except a marked difference between the consistency of the two areas. Crampton (1928), while describing in general the eulabium, mentum, submentum, and gular regions of insects, has mentioned that in such cases (where the gular and the submental regions are not marked off from each other by any suture) the gular and the submental regions are nearly areas of the gulomental plate which becomes secondarily differentiated into the gular and the submental regions. Thus the area above the metatentorinæ and below the gulomental suture forms the submentum.

The glossæ and paraglossæ are reduced and fused into each other to form a median structure pointed at its apex, the ligula. The lateral portions of the ligula may represent the paraglossæ while the median portion represents the glossæ. But Crampton (1928) describes that the median chitinisation of the ligula, called the glossa in the beetles, is the mediglossa and in reality has nothing to do with the true glossæ which probably atrophied in the beetles (the glossæ begin to atrophy even in such primitive insects as the grasshoppers; and the glossæ are completely lost in such forms as the Dermaptera which are very like the ancestors of beetles in many anatomical features). The mesial surface of the ligula is provided with a number of sensory patches. The ligula is not a free part of the labium but is embedded

in a thin membrane connecting the two cephalic parts of the hypopharynx with the labium. On each lateral side of the prementum is present a distinct sclerite, the palpiger, which bears a three-segmented labial palpus. The first segment of the palpus is the longest of all, the second is a bit shorter than the first, but both of them are oval. The third segment is very small and looks like a short oval cephalic outgrowth of the second segment. The second segment bears numerous short and long hair on all its surfaces.

(*e*) *Hypopharynx* (Text-Fig. 9).—The large membranous hypopharnyx arises from the floor of the mouth adnate to the posterior wall of the labium and the anterior margin of the clypeus. The hypopharynx is U-shaped and consists mainly of two parts—the cephalic and the caudal. The caudal part



TEXT-FIG. 9. Hypopharynx, dorsal view.

forms the main portion of the body of the pharynx and articulates with the labium and the clypeus. The lateral sides of the caudal part send out lateral extensions supported by two vertical chitinous bars, the hypopharyngeal bars of Dorsey (1943). The lateral extensions are separated from the main body by means of two oblique sutures. The cephalic ends of the hypopharyngeal bars arise from two lateral invaginations of the labial suture on the ventral side of the labium and become funnel-like at their caudal ends. A short median transverse chitinous bar, the interhypopharyngeal bar, connects the two hypopharyngeal bars. Supporting the hypopharynx in its middle and running on its dorsal side is another vertical bar, the midhypopharyngeal bar, which joins the interhypopharyngeal bar by its caudal end. Each arm

of the U-shaped hypopharynx cephalad bears a brush-like structure having soft hair on its mesial margin, covering such portion as extends up to the mentum. At the lateral side of each brush is a semicircular, much chitinised lateral sclerite. The junction of the two hypopharyngeal arms on its ventral side also carries a small oval brush-like structure with soft hair. The ligula of the labium lies embedded in a thin membrane extending between the two hypopharyngeal arms.

(*f*) *Tentorium.—H. bucephalus* bears a very weak and non-chitinised endoskeleton. The posterior tentorial arms, which are weaker than the anterior ones, unite to form the carpotentorium. These two arms open out to the exterior by two invaginations, the metatentorinæ, situated at the caudal ends of the paracoila on either side. From the lateral ends of carpotentorium are given out the two tentorial arms which extend outwards and bifurcate at their caudal ends to meet the dorsal wall of the head capsule. Each anterior tentorial arm opens to the exterior through a furrow, the pretentorina, situated at the caudal end of each epicranial suture. Each anterior tentorial arm gives out a long tendon, the œsotendon, which broadens distally and is directed ventrad.

V. Summary

There is no detailed record of the morphology of the head capsule and mouth parts of any Indian species of the subfamily Coprinæ. The present work deals with the morphology of the head capsule and mouth parts of *Heliocopris bucephalus* Fabre. The following features are noteworthy:—

1. The head capsule displays sexual dimorphism by means of a frontal horn in the male and a frontal carina at the corresponding position in the female. The occiput and the occipital sutures are indistinguishable. A deep cavity on the frontal side of the frons into which fits the clypeolabrum is present.

2. The antennæ are situated on the ventral side of the epicranial suture.

3. The clypeus and the frons are not in the same level, the caudal margin of the preclypeus is articulated with the hypopharynx, and hence, an epistomial suture is absent. The clypeal and labral epipharynx is marked with ridges.

4. The mandibles are masticatory and grinding organs.

5. The maxillæ are soft with membranous distigalea and bear no teeth for biting.

6. The labium is reduced because of the greater development of the gula. The submentum is marked off from the gula by the position of the metatentorinæ. The labial suture is distinct only in its lateral portions.

7. The hypopharynx is U-shaped and membranous and bears three brush-like structures.

8. The endoskeleton is very weak and non-chitinous.

VI. ACKNOWLEDGEMENTS

We express our gratitude to Shri Bhim Sen, Principal, Government College, Ajmer, for research facilities.

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* Quoted from Khatib.

VIII. LETTERING

abap .. abductor apodeme.
adap .. adductor apodeme.
anc .. antacorium.
an.scl .. antennal sclerite.
ant .. antenna.
bg .. basigalea.

bscl .. basal sclerite.
ca .. cardo.
cap .. cardostipital apodeme.
cbr .. cephalic brush.
ce .. compound eye.
clls .. clypeolabral suture.
clp .. clypealia.
cls .. clypeal suture.
cr .. cardostipital ridge.
cubr .. caudal brush.
dg .. distigalea.
epr .. epipharyngeal ridge.
eps .. epicranial suture.
eust .. eustipes.
fl .. flagellum.
fr .. frons.
frc .. frontal carina.
frh .. frontal horn.
g .. gula.
ge .. gena.
gh .. gular hair.
gms .. gulomental suture.
gr .. galeal ridge.
gs .. gular suture.
h .. hair.
hy .. hypopharynx.
hy.b .. hypopharyngeal bar.
i hy.b .. interhypopharyngeal bar.
ip .. inner part of prostheca.
l .. labium.
la .. lacinia.
lb .. labrum.
lepr .. labro-epipharyngeal ridge.
lext .. lateral extension of the hypopharynx.
lg .. ligula.
lp .. labial palpus.
ls .. labial suture.
l scl h .. lateral sclerite of the hypopharnyx.
l.scl m .. lateral sclerite of the mandible.
m .. mentum.
max .. maxilla.
max.p .. maxillary palpus.
m.hy.b .. midhypopharyngeal bar.
mlb. .. mesolabrum.
m.scl .. mesial sclerite of the mandible.
mt .. metatentoriana.
obr .. oblique ridge on the epipharynx.
of .. occipital foramen.
ol .. oculata.
op .. outer part of the prostheca.
pd .. pedicel.
pf .. palpifer.
pge .. postgena.
pl .. paracoila.
pmt .. prementum.
pn .. pretentorina.
po .. postocciput.
pocl .. postclypeus.
pos .. postoccipital suture.
pp .. palpiger.
pr .. precoila.
prap .. promotor apodeme.
prcl .. preclypeus.
pst .. parastipes.
prthr .. prosthecal ridge.
pta .. postartis.
ptc .. postcoila.
py .. pretartis.
r .. ridge.
s .. suture.
sas .. subantennal suture.
sc .. scape.
sp .. sensory patches.
smt .. submentum.
st .. stipes.
tp .. terminal part of the mandible.
trb .. transverse bar.
v .. vertex.

GROWTH FACTOR REQUIREMENTS OF *PIRICULARIA* SPP. AND *SCLEROTIUM ORYZÆ**

By S. Suryanarayanan

(*University Botany Laboratory, Madras*)

[Communicated by Professor T. S. Sadasivan, D.Sc. (Lond.), F.N.I., F.A.Sc.]

Ever since Schopfer (1934) demonstrated the necessity of an external supply of thiamine to *Phycomyces blakesleeanus*, a great many fungi have been studied for their growth factor requirements and the literature in this regard has been reviewed by Robbins and Kavanagh (1942), Schopfer (1943), Fries (1948), Hawker (1950) and Lilly and Barnett (1951). However, work of this nature has received little attention in India except for studies on a few fungi, *viz.*, *Chætomium brasiliensis* (Basu, 1952), *Corticium microsclerotia* (Mathew, 1953) and *Pellicularia koleroga* (Mathew, 1952, 1954). A preliminary screening of several crop-infecting fungi for their vitamin deficiencies in this laboratory indicated quite a few of them to be heterotrophic and these have been reported (Sadasivan and Subramanian, 1954). The present paper deals with the more elaborate studies conducted on different species and isolates of *Piricularia* and *Sclerotium oryzæ* for their vitamin deficiencies. Of the few species of *Piricularia*, only *Piricularia oryzæ* has been studied for its growth factor needs by other workers. The vitamin deficiencies of other species and isolates of *Piricularia* from different hosts and of *Sclerotium oryzæ* have not been reported.

The beneficial effect of the addition of the vitamin-like 'oryzanin' to the nutrient medium was observed to accelerate the formation and germination of conidia of *Piricularia oryzæ* (Ito and Terui, 1931). Working under more exacting conditions and with synthetic vitamins Leaver *et al.* (1947) indicated the heterotrophy of this fungus to thiamine and biotin. More recent work in Japan with this organism showed it to be mainly biotin deficient, thiamine being complementary (Otani, 1952, Tanaka and Katsuki, 1953). However, earlier studies made by the author with a strain of this fungus isolated in India revealed mainly thiamine deficiency, biotin being complementary (Appa Rao *et al.*, 1955).

Apart from the vitamin studies of this fungus little work has been done on its nutritional physiology. Little or no information is available regard-

* Part of thesis approved for the Degree of Doctor of Philosophy of the University of Madras, 1956.

ing the physiological behaviour of the different species and isolates of *Piricularia* and *Sclerotium oryzæ* especially from the aspect of their growth factor requirements. Even though knowledge is still lacking on the exact significance of growth factors in host-parasite relationship, their importance in fungal physiology cannot be overlooked and to quote Foster (1949), "Much of the earlier work on the ability of an organism to utilize various forms of nitrogen nowadays must be re-examined in the light of accumulated knowledge of vitamins and growth factors. The inability of an organism to develop on a form of nitrogen in a synthetic medium does not necessarily mean that the particular nitrogen compound was not assimilable; it may rather mean that certain vitamins also essential for growth were lacking and that their need was imperceived in 'pre-vitamin' times."

Material and Methods

The methods of investigation in this field are exacting. Hence, the factors that tend to vitiate the results were kept at the minimum by strictly adhering to the codes of procedure indicated by Robbins and Kavanagh (1942). Slight improvements and modifications were made as and when necessary.

Source and maintenance of cultures.—Most of the cultures were kindly supplied by the Government Mycologist, Agricultural Research Institute, Coimbatore. Others were either isolated by the author or obtained from Centraalbureau Voor Schimmelcultures, Baarn, Holland. The details of the source and their maintenance are given below:

The above cultures were subcultured once a fortnight.

Glassware, water, chemicals and vitamins.—Pyrex Erlenmeyer flasks of 250 ml. capacity and test-tubes of the same brand were employed. Cleaning of glassware was done after Mathew (1953). Metal distilled water redistilled in a Pyrex still was used in earlier experiments and later, water distilled in an all-glass Pyrex electric still was found satisfactory. Chemicals of utmost purity, vitamins and their components referred to by Mathew (1953) were used.

Basal medium.—The basal medium consisted of: $NaNO_3$: 2·5 g., KH_2PO_4: 1·0 g., $MgSO_4.7H_2O$: 0·5 g., Sucrose: 5·0 g. and glass distilled water to make 1,000 ml. supplemented with 0·1 ml. of a trace element solution of Robbins (1939 *b*), containing B, Cu, Fe, Mn, Mo and Zn. An aliquot of 25 ml. of this, pipetted out in each flask or 5 ml. in each tube was maintained throughout the investigation. In studies, on the influence of different nitrogen and carbon compounds, substitution to the above medium was always made on an equivalent of nitrogen or carbon. The pH was adjusted

Name of the fungus	Host	Maintenance	Source
Piricularia oryzæ Cav. (Strain 1)	*Oryza sativa*	2% P.D. agar	Govt. Mycologist, Coimbatore
P. oryzæ (Strain 2)	,,	Sterilized host bits	Isolated by author
P. setariæ Nisikado (Strain 1)	*Setaria italica*	2% P.D. agar	Govt. Mycologist, Coimbatore
P. setariæ (*Strain* 2)	,,	Sterilized host bits	Isolated by author
P. zingiberi Nisikado	*Zingiber* sp.	2% P.D. agar	C. B. S., Holland.
Piricularia sp. (Strain 1)	*Eleusine coracana*	,,	Govt. Mycologist, Coimbatore
Piricularia sp. (Strain 2)	,,	Sterilized host bits	Isolated by author
Piricularia sp.	*Triticum vulgare*	2% P.D. agar	Govt. Mycologist, Coimbatore
Piricularia sp.	*Brachiaria mutica*	,,	,,
Piricularia sp.	*Leersia hexandra*	,,	,,
Sclerotium oryzæ	*Oryza sativa*	,,	,,

P.D.: Potato-dextrose.

to 6·0 before autoclaving with 6 N.NaOH or 6 N.HCl with the aid of a Hellige comparator. Agar when used was purified with 5% aqueous pyridine (Robbins, 1939 *a*). Vitamins were added before autoclaving. Both solid and liquid media were sterilized at 15 lb. pressure for 15 minutes.

Aerial mycelium without any agar adhering to it formed the inoculum for all *Piricularia* spp. and a single sclerotium was inoculated in the case of *Sclerotium oryzæ*. In later experiments the method of Wikén *et al.* (1951) was found to be more satisfactory. This consists in growing the fungus for six days in the liquid medium described above with sub-optimal doses of thiamine and biotin and macerating the fungal mat with glass beads in sterile glass distilled water in a Pyrex boiling tube. A loopful of this

mycelial suspension was added to each flask or tube as the case may be. This procedure had the definite advantage of tending to greatly reduce the variation between replicates as well as minimizing the carry over of growth factors along with the inoculum. All cultures were incubated at room temperature (29 ± 3° C.).

Growth determination and data.—Growth in test-tubes, either on solid or liquid, media were visually rated. Mats from liquid cultures were harvested by filtering through Gooch crucibles, washed thoroughly with distilled water, dried over filter-papers at 55–60° C. for 48 hours, cooled in a desiccator and weighed. The mean value of three replicates have been presented along with statistical deductions following the methods of Paterson (1939) and Morony (1951).

Experimental

Preliminary screening of *Piricularia oryzæ*, *P. setariæ*, *P. zingiberi* and isolates of *Piricularia* from *Eleusine coracana*, *Triticum vulgare*, *Brachiaria mutica*, *Leersia hexandra* and *Sclerotium oryzæ* on an asperagine-sucrose medium solidified with 2% pyridine washed agar showed them to be incapable of growth on the vitamin-free synthetic medium, thereby indicating vitamin deficiency. The following experiments represent a closer examination of the nature and type of heterotrophy of these organisms.

1. *Vitamin deficiency of species and isolates of* Piricularia

Vitamin deficiencies of the six *Piricularia* referred *supra* were evaluated using the liquid medium (*vide* 'Materials and Methods') by having a vitamin mixture (+ All) of the following composition: (1) thiamine 5·0 μg., (2) biotin 2·5 μg., (3) pyridoxine 5·0 μg., (4) nicotinic acid 5·0 μg., (5) inositol 1·0 μg., (6) *p*-amino benzoic acid 5·0 μg., (7) riboflavin 2·5 μg. and (8) pantothenic acid 5·0 μg. Flasks without added vitamins served as controls. The effect of excluding any one vitamin at a time from the mixture (+ All) was studied. The trial was conducted in triplicates for each treatment.

A pin-head size of aerial mycelium removed from a ten-day old potato-dextrose slant formed the inoculum. The cultures were incubated for 15 days, the mats harvested and weighed (*vide* 'Methods').

The data are presented in Table I.

Results.—In the controls, without added vitamins, slight growth occurred with all the seven isolates which amounted to as little as 2% in the case of the isolate from *Leersia hexandra* to as much as 19% with that from *Brachiaria mutica*. Thiamine, when omitted from the vitamin mixture (+ All) decreased

TABLE I

Effect of omitting a single vitamin from a mixture (+All) on the growth of Piricularia *spp.*

Treatments	Mat weight (mg.)						
				Isolates from			
	A *P. oryzæ*	B *P. setariæ*	C *P. zingiberi*	D *E. coracana*	E *T. vulgare*	F *B. mutica*	G *L. hexandra*
1 Control ..	2·5	5·7	1·1	5·9	3·1	4·2	0·4
2 — Thiamine ..	5·1	7·3	0·8	3·9	4·8	11·2	1·1
3 — Biotin ..	24·1	35·1	26·6	17·1	15·9	20·7	16·3
4 — Pyridoxine ..	33·0	50·1	37·1	29·4	19·7	30·0	35·3
5 — Nicotinic Acid	33·7	50·5	34·6	30·4	20·8	28·5	31·3
6 — Inositol ..	29·6	45·7	34·7	29·5	20·7	29·5	31·3
7 — Pantothenic Acid	31·2	47·1	34·4	28·8	19·1	31·0	33·3
8 — *p*-A.B. Acid ..	35·4	51·4	35·5	32·4	19·0	31·7	35·1
9 — Riboflavin ..	32·7	43·0	37·3	29·7	17·9	29·4	26·6
10 + All ..	33·2	43·3	34·9	31·7	20·0	29·1	25·9
Significance by F. test ..	Yes	Yes	Yes	Yes	Yes	Yes	Yes
C.D. at 1% level ..	4·1	12·8	4·3	3·3	3·0	2·6	4·1

Conclusions

A: 8 5 10 4 9 7 6 3 2 1

B: 8 5 4 7 6 10 9 3 2 1

C: 9 4 8 10 6 5 7 3 1 2

D: 8 10 5 9 6 4 7 3 1 2

E: 5 6 10 4 7 8 9 3 2 1

F: 8 7 4 6 9 10 5 3 2 1

G: 4 8 7 5 6 9 10 3 2 1

growth considerably. This reduction in growth was equal, slightly more, or less than that obtained in the controls. Exclusion of biotin, however, brought down growth only to a smaller extent. Withholding of other vitamins had no retarding effect on growth of the organisms. Even though withdrawal of certain vitamins other than thiamine and biotin tended to increase growth with certain isolates, the effect was significantly marked only in the case of the isolate from *Leersia hexandra* where pyridoxine, nicotinic acid, inositol, pantothenic acid and *p*-amino benzoic acid proved to be definitely toxic to growth.

2. *Effect of thiamine and biotin added singly on the growth of* Piricularia *spp.*

Experiment 1 has shown that excluding thiamine from the (+ All) mixture, markedly reduced growth of all *Piricularia* spp. while withholding of biotin decreased growth only to a slight extent.

The effect of thiamine and biotin added singly to the medium on growth of these fungi was next studied. Thiamine at 1·0 μg. and biotin at 0·1 μg. levels were added to 5 ml. of the standard medium in Pyrex test-tubes. The tubes were inoculated with a loopful of mycelial suspension (*vide* 'Methods') and the growth of the different fungi was visually rated on the 15th day and the data are presented in Table II.

TABLE II

Growth of different isolates of Piricularia *spp. with thiamine and biotin*

Name of the fungus	Treatments			
	Control	Thiamine 1 μg.	Biotin 0·1 μg.	Thiamine 1 μg.+ Biotin 0·01 μg.
P. oryzæ ..	−	+	−	++++
P. setariæ ..	−	+	−	++++
P. zingiberi ..	−	+	−	++++
Piricularia sp. on *Eleusine coracana*	−	+	−	++++
Piricularia sp. on *Triticum vulgare*	−	+	−	++++
Piricularia sp. on *Brachiaria mutica*	−	+	−	++++

− Negligible growth.
\+ Slight growth.
++++ Full growth.

Results.—All the different species and isolates of *Piricularia* made negligible growth in the controls or with biotin alone. Very slight growth was observed with thiamine. Full growth of the fungi occurred only when both thiamine and biotin were present in the medium.

3. *Vitamin deficiency of* Sclerotium oryzæ

The vitamin deficiency of this fungus was investigated on slants of purified agar and the results are presented in Table III and Plate XXI.

TABLE III

Vitamin deficiency of Sclerotium oryzæ

Treatments	Growth ratings		Sclerotial production	
	6th day	12th day	6th day	12th day
Control ..	−	−	−	−
− Thiamine ..	−	−	−	−
− Biotin ..	++	++++	++	++++
−Pyridoxine ..	++	++++	++	++++
− Nicotinic acid ..	++	++++	++	++++
− Inositol ..	++	++++	++	++++
− Pantothenic acid ..	++	++++	++	++++
− *p*-Amino benzoic acid ..	++	++++	++	++++
− Riboflavin ..	++	++++	++	++++
+ All ..	++	++++	++	++++

− Negligible.
++ Fair.
++++ Full.

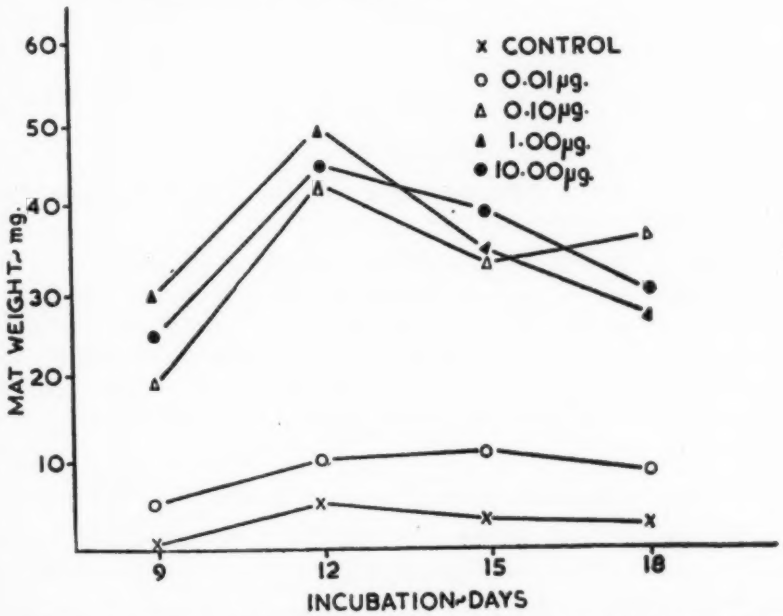
Results.—Growth was negligible in the control and in the thiamine omitted series (Plate XXI). Withdrawal of other vitamins did not affect growth or sclerotial reduction. Sclerotia were observed to form from the sixth day after inoculation. The different vitamins did not apparently influence the amount of sclerotial production.

4. *Levels of thiamine and biotin as a function of time for* Piricularia *spp.*

A priori all the species and isolates of *Piricularia* and *Sclerotium oryzæ* appear to be heterotrophic to thiamine and biotin and thiamine respectively. However, the nature of such a heterotrophy needs to be considered by a study of the effect of different levels of the concerned vitamins as a function of time. Besides, the experiment had in it the object of fixing an optimum period of incubation for the fungi in question and to be followed in further investigation.

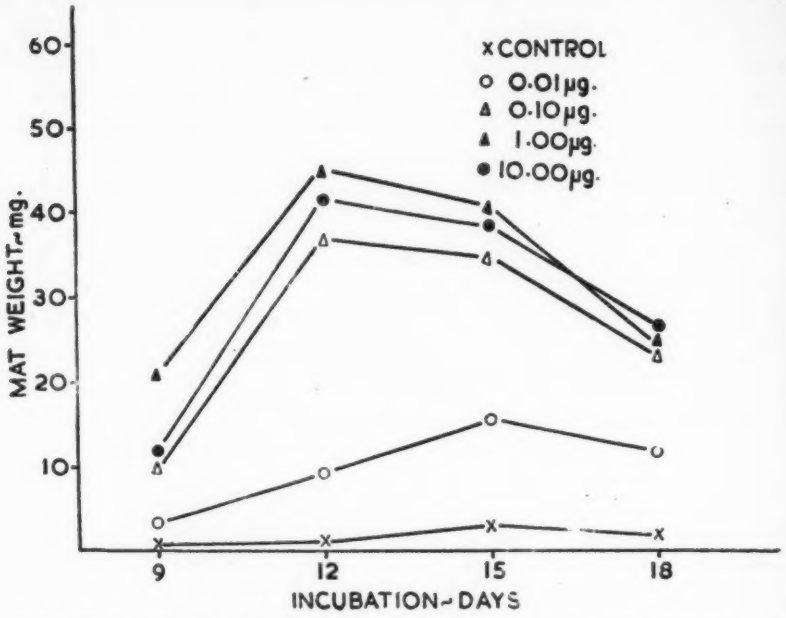
Methods already mentioned in previous experiments were followed. Thiamine and biotin at 0·01 μg., 0·10 μg., 1·00 μg. and 10·00 μg. were added to each flask. The controls had no added vitamins. Triplicate cultures in each treatment were harvested at periodic intervals of three days from the 9th to 18th day after inoculation.

The data are presented in Text-Figs. 1–6.



TEXT-FIG. 1. Growth of *Piricularia oryzæ* with various levels of thiamine and biotin at different incubation periods.

Results.—Slight or negligible growth occurred in the controls throughout the different incubation periods. With 0·01 μg. of the vitamins, growth was accelerated but significant growth was obtained only with 0·1, 1·0,



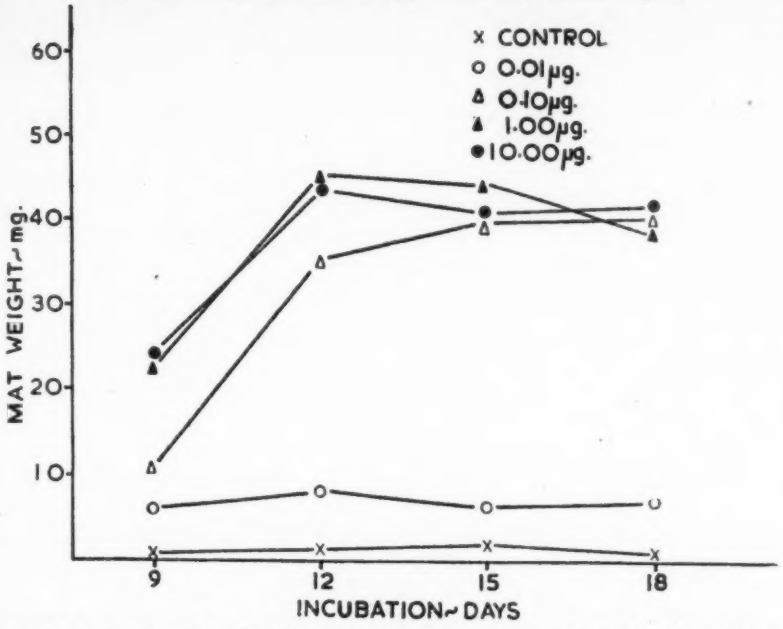
TEXT-FIG. 2. Growth of *Piricularia setariæ* with various levels of thiamine and biotin at different incubation periods.

10·0 µg. of the vitamins. Maximum growth of all isolates took place with 1·0 µg. level of both the vitamins on the 12th day, growth beginning to decline thereafter indicating autolysis. Active growth of the fungi was evident only between the 9th and 12th day and levels of vitamins higher than 0·1 µg. were required to promote significant growth. Incubating the controls upto 18 days did not materially benefit the organisms to any advantage.

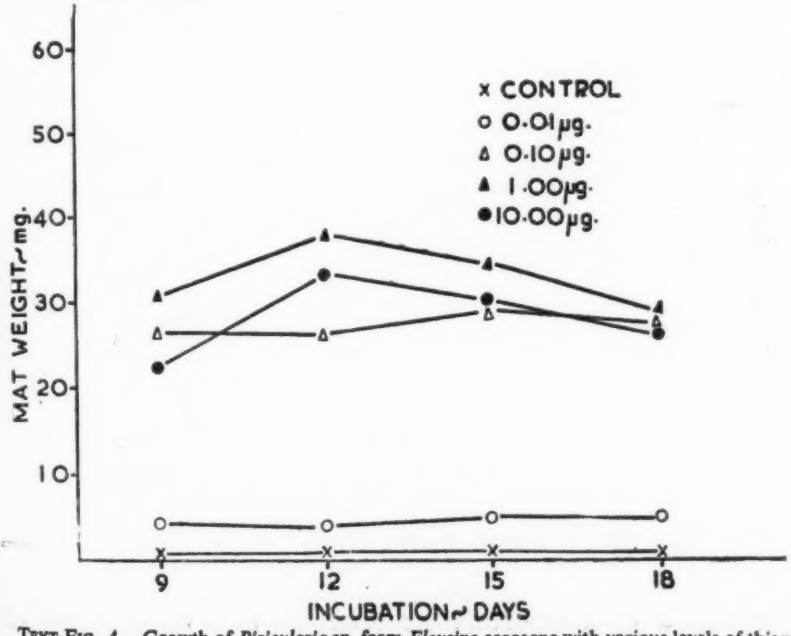
5. *Levels of thiamine as a function of time for* Sclerotium oryzæ

Experiment 3 has shown this organism to be heterotrophic only to thiamine. The object and experimental procedure followed was the same as the preceding one.

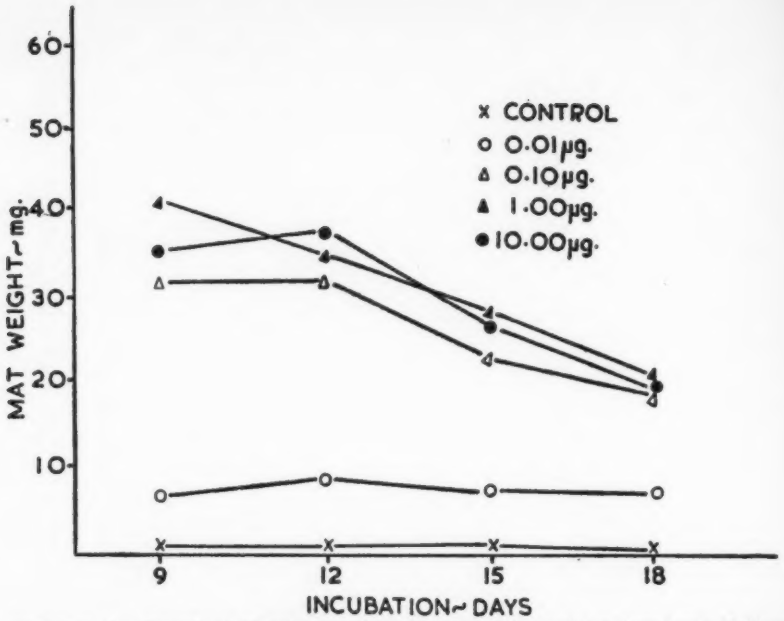
Single sclerotium was used for inoculation. Mats were harvested at intervals of 6 days and the incubation period ranged from 6 to 24 days. The amounts of thiamine added per flask were: none, 0·001 µg., 0·01 µg., 0·10 µg. and 10·0 µg.



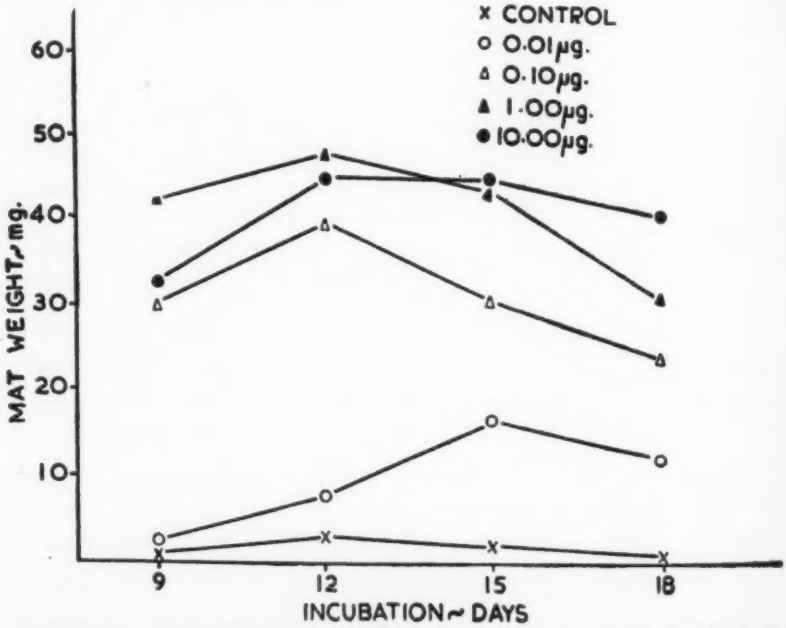
TEXT-FIG. 3. Growth of *Piricularia zingiberi* with various levels of thiamine and biotin at different incubation periods.



TEXT-FIG. 4. Growth of *Piricularia* sp. from *Eleusine coracana* with various levels of thiamine and biotin at different incubation periods.



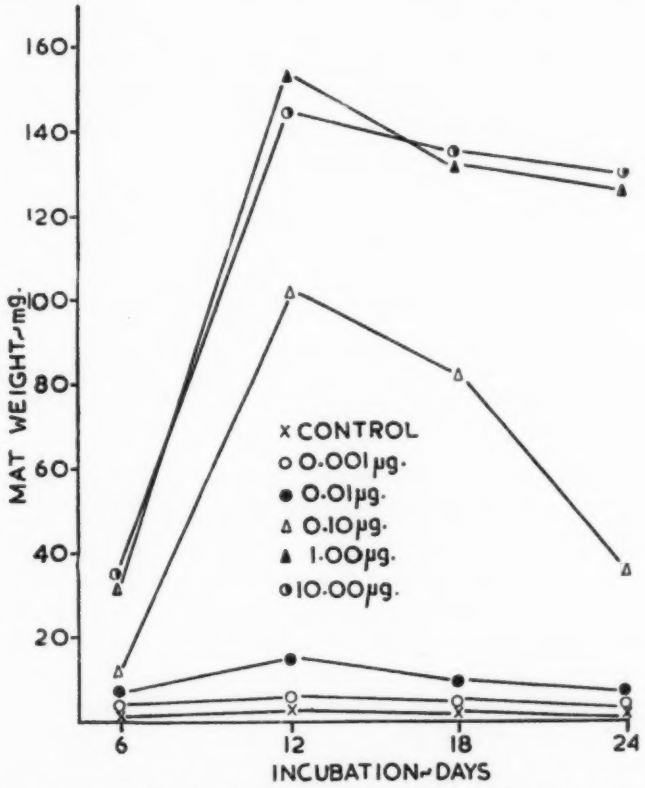
TEXT-FIG. 5. Growth of *Piricularia* sp. from *Triticum vulgare* with various levels of thiamine and biotin at different incubation periods.



TEXT-FIG. 6. Growth of *Piricularia* sp. from *Brachiaria mutica* with various levels of thiamine and biotin at different incubation periods.

The data are presented in Text-Fig. 7.

Results.—Growth was negligible in the controls at all incubation periods. Addition of 0·001 μg. and 0·01 μg. of thiamine did not bring about any change. Growth of the fungus with 0·1 μg. of the vitamin was negligible at the end of 6 days while this level promoted 66% growth by 12 days. Significant growth occurred even by 6 days with 1·0 μg. and 10·0 μg. of the vitamin. Maximum growth was reached by 12 days with 1·0 μg. and 10·0 μg. of the vitamin. Autolysis had set in after 12 days with all levels of the vitamin.



TEXT-FIG. 7. Growth of *Sclerotium oryzæ* with various levels of thiamine at different incubation periods.

6. *Optimum level of thiamine and biotin for* Piricularia *spp.*

Experiment 4 indicated that the optimum period of incubation for all the three species of *Piricularia, viz., P. oryzæ, P. setariæ, P. zingiberi* and

the isolate from *Brachiaria mutica* was about 12 days and that thiamine and biotin in excess of 0·01 μg. per flask were needed to promote significant growth of all the six fungi. However, the optimum period was only 9 days (as evident from the statistical treatment of the data) for the isolates from *Eleusine coracana* and *Triticum vulgare*.

In order to determine the optimum levels of thiamine and biotin for these fungi, various levels of thiamine, ranging from 0·01 μg. to 5·0 μg. together with biotin at 0·001 μg., 0·01 μg. and 0·1 μg. levels were added per flask. The flasks were inoculated as before and incubated for 12 or 9 days, as the case may be.

The data are presented in Table IV.

Results.—The results in general indicate 1·0 μg. of thiamine and 0·01 μg. of biotin to be the optimum, irrespective of the species or isolate.

7. *Optimum level of thiamine for* Sclerotium oryzæ

The experimental procedure was the same as that of the previous experiment. Thiamine levels ranging from 0·01 μg. to 5·0 μg. in the order of 0·01 μg., 0·10 μg., 1·00 μg., 2·00 μg. and 5·00 μg. were tried as this fungus has been shown to be heterotrophic only to thiamine (*vide* Expt. 3).

The data are presented in Text-Fig. 8.

Results.—The optimum amount of thiamine was found to be 1·0 μg. per flask.

8. *Vitamin deficiencies of pathogenic and non-pathogenic isolates of* Piricularia oryzæ, P. setariæ *and* Piricularia *sp. from* Eleusine coracana

The isolates of *Piricularia oryzæ*, *P. setariæ* and *Piricularia* sp. from *Eleusine coracana* mentioned in the foregoing experiments were maintained on 2% potato-dextrose agar. Repeated inoculation trials showed these isolates to have lost their virulence and not to infect their respective hosts, *viz.*, *Oryza sativa*, *Setaria italica* and *Eleusine coracana*. It was, therefore, thought worthwhile to study if qualitative differences would exist in the vitamin requirements of fresh and old isolates. The experiment had also another end in view, *i.e.*, if cultures maintained on sterilized host bits would differ in their vitamin needs from those on media like potato-dextrose agar.

A number of single spore isolates of *P. oryzæ*, *P. setariæ* and *Piricularia* sp. from *Eleusine coracana* were made and maintained on sterilized host bits as well as on potato-dextrose agar. They were tested for their virulence by spraying spore suspensions and found to infect their respective

TABLE IV

Growth of Piricularia *spp. with different levels of thiamine and biotin*

Levels of vitamins in μg./flask			Mat weight (mg.)					
						Isolates from		
	Thiamine	Biotin	A *P. oryzæ*	B *P. setariæ*	C *P. zingiberi*	D *E. coracana*	E *T. vulgare*	F *B. mutica*
1	0·01	0·001	4·7	9·1	6·3	6·8	7·5	5·9
2	0·10	0·001	19·7	25·7	33·3	35·4	23·9	14·7
3	1·00	0·001	39·1	25·1	31·2	37·5	26·6	16·5
4	2·00	0·001	35·2	25·2	33·3	36·5	26·9	19·1
5	5·00	0·001	43·9	26·6	29·9	40·2	23·1	20·7
6	0·01	0·01	6·7	10·6	5·7	6·7	6·1	6·8
7	0·10	0·01	36·3	24·2	30·0	36·8	27·2	24·1
8	1·00	0·01	44·3	37·7	45·3	42·1	33·7	34·1
9	2·00	0·01	43·3	35·3	44·1	36·5	29·9	34·5
10	5·00	0·01	40·2	35·6	41·5	39·3	27·5	30·9
11	0·01	0·10	6·3	10·3	6·0	5·8	7·8	9·6
12	0·10	0·10	24·9	32·1	34·9	37·1	26·7	24·1
13	1·00	0·10	44·3	31·8	43·9	42·8	28·8	33·9
14	2·00	0·10	37·5	36·5	41·1	42·3	27·2	32·9
15	5·00	0·10	38·3	36·9	45·1	41·2	26·6	35·1
Significance by F. test			Yes	Yes	Yes	Yes	Yes	Yes
C.D. at 1% level ..			3·5	5·8	5·8	1·6	3·5	7·4

Conclusions

A: 8 13 5 9 10 3 15 14 7 4 12 2 6 11 1

B: 8 15 14 10 9 12 13 5 2 4 3 7 6 11 1

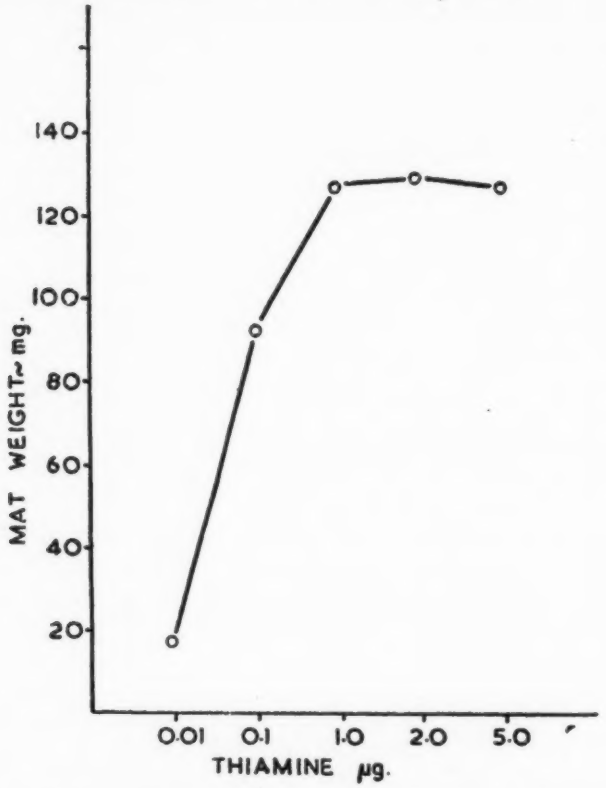
C: 8 15 9 13 10 14 12 2 4 3 7 5 1 11 6

D: 13 14 8 15 5 10 3 12 7 4 9 2 1 6 11

E: 8 9 13 12 7 14 4 12 3 15 2 5 11 1 6

F: 15 9 8 13 14 10 8 12 5 4 3 2 11 6 1

hosts. These isolates have been termed as *P. oryzæ* (Strain 2), *P. setariæ* (strain 2) and *Piricularia* sp. from *E. coracana* (Strain 2).



TEXT-FIG. 8. Growth of *Sclerotium oryzæ* with different levels of thiamine.

Comparative studies were made on the vitamin deficiencies of these pathogenic strains together with the non-pathogenic strains used in the earlier part of this investigation, hereinafter to be called as *P. oryzæ* (Strain 1), *P. setariæ* (Strain 1), and *Piricularia* sp. from *E. coracana* (Strain 1). These isolates were studied for their vitamin deficiencies in liquid cultures in test-tubes. Only four of the vitamins for which more often fungi are known to be deficient, *viz.*, thiamine, biotin, pyridoxine and inositol were used in this study. The treatments comprised of: no vitamins, thiamine 1·0 µg., biotin 0·1 µg., thiamine 1·0 µg. + biotin 0·1 µg., and thiamine 1·0 µg. + biotin 0·1 µg. + pyridoxine 1·0 µg. + inositol 25 µg. per tube.

Growth ratings were made visually after 12 days incubation and the results are presented in Table V.

TABLE V

Growth of non-pathogenic and pathogenic isolates of Piricularia oryzæ, P. setariæ *and* Piricularia *sp. on* Eleusine coracana *with different vitamins*

Name of fungus	Nature of the isolate	Vitamins				
		None	T	B	T+B	T+B+Py+I
P. oryzæ (Strain 1)	Non-pathogenic	−	+	−	++++	++++
P. oryzæ (Strain 2)	Pathogenic	−	+	−	++++	++++
P. setariæ (Strain 1)	Non-pathogenic	−	+	−	++++	++++
P. setariæ (Strain 2)	Pathogenic	−	+	−	++++	++++
Piricularia sp. on *E. coracana* (Strain 1)	Non-pathogenic	−	+	−	++++	++++
Piricularia sp. on *E. coracana* (Strain 2	Pathogenic	−	+	−	++++	++++

− Negligible growth.
\+ Slight growth.
++++ Full growth.
T: Thiamine.
B: Biotin.
Py: Pyridoxine.
I: Inositol.

Results.—The results indicated that all the isolates were heterotrophic only to thiamine and biotin regardless of their time of isolation and maintenance on either potato-dextrose agar or sterilized host bits.

9. *Influence of the forms of nitrogen on the vitamin deficiency of non-pathogenic and pathogenic isolates of* Piricularia

It has been known that the vitamin requirements of a fungus may change with a change in the nitrogen source. The object of this experi-

ment was to compare the vitamin needs of non-pathogenic and pathogenic isolates of *P. oryzæ*, *P. setariæ* and *Piricularia* sp. from *Eleusine coracana* with different nitrogen sources.

The experimental procedure, the nature and amounts of vitamins added were the same as in the preceding experiment. Ten different nitrogen compounds were used representing the inorganic nitrate and ammonium nitrogen and organic nitrogen.

The data are presented in Table VI.

Results.—Both the pathogenic and non-pathogenic isolates of *Piricularia* behaved alike not only in their vitamin needs but also in their ability to utilize the different forms of nitrogen. They were heterotrophic to thiamine and biotin regardless of the nitrogen source. In general, these organisms were found to utilize organic nitrogen and inorganic nitrate nitrogen. Inorganic ammonium nitrogen was less satisfactory and nitrite inhibited growth completely. Presence of pyridoxine and inositol along with thiamine and biotin did not enable any of the fungi to better utilize the inorganic ammonium nitrogen.

10. *Influence of the source of carbon on the vitamin deficiencies of non-pathogenic and pathogenic isolates of* Piricularia

Following the procedure of Expt. 8, the influence of different carbon sources on the vitamin deficiencies of pathogenic and non-pathogenic isolates of *Piricularia* were studied by replacing sucrose of the standard medium with 9 different sugars on an equivalent basis.

The data are presented in Table VII.

Results.—All the three isolates, irrespective of their virulence, were heterotrophic only to thiamine and biotin with all the sources of carbon. In general, addition of pyridoxine or inositol was not beneficial to any of the fungi. All the six isolates practically made no growth with mannitol, dulcitol and glycerol. The pathogenic strains were found to utilize the sugars better than the non-pathogenic strains, though their ability to utilize the different carbon sources did not differ. Glucose, fructose, sucrose and maltose appeared to be better sources of carbon than the other sugars for all the three fungi. However, *P. setariæ* and *Piricularia* sp. from *E. coracana* could use with equal facility lactose and xylose respectively.

11. *Influence of pH of the medium on the vitamin needs of non-pathogenic and pathogenic isolates of* Piricularia

Studying the influence of pH on the vitamin deficiencies of non-pathogenic and pathogenic isolates of *Piricularia* on the same lines as Expts. 9

TABLE VI

Growth factor requirements of non-pathogenic (NP) and pathogenic (P) isolates of Piricularia oryzæ *(PO),* P. setariæ *(PS) and* Piricularia *sp. from* Eleusine coracana *(EC) with different nitrogen sources*

Treatments ..	Control						Thiamine						Biotin						Thiamine + Biotin						+ All					
Nitrogen Source	PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
Sodium nitrate ..	0	0	0	0	0	–	1	2	2	1	2	2	1	1	0	1	0	–	4	4	4	4	3	4	4	4	4	4	4	4
Potassium nitrate ..	0	0	0	0	0	–	1	2	2	1	2	2	1	1	0	0	1	–	4	4	4	4	4	4	4	4	4	4	4	4
Potassium nitrite ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonium nitrate ..	0	0	–	0	–	1	1	1*	2	2	2	2	1	1	–	0	2	–	2	2	2	3	4	3	2	2	3	3	4	3
Ammonium chloride ..	–	–	–	–	–	–	1	1	2	1	2	2	–	–	–	–	–	–	2	2	2	2	3	2	2	2	2	2	3	2
Ammonium sulphate ..	1	1	1	0	1	1	1*	2	2	1	1	2	1	1	–	–	–	1	2	2*	2	2	3	2	2	2*	2	2	3	2
Ammonium oxalate ..	–	–	–	0	0	1*	1	1	2	1*	2	2	–	–	–	0	0	2	3	3	3	4	4	4	3	3	3	4	4	4
Ammonium tartrate ..	–	–	–	0	–	1	1	1	2	2	2	2	1	–	–	–	1	–	3	3	4	4	4	3	3	3	4	4	4	3
Urea ..	0	–	0	0	0	–	1	1*	1	2	2	2	–	–	–	0	–	–	3	3	3	3	3	3	3	3	3	3	3	3
Asparagine ..	0	–	–	–	–	–	1	1*	1	2	2	2	–	1	–	–	1	1	4	4	3	4	4	4	4	4	3	4	4	4

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

0: Nil.
–: Negligible.
1: Very slight.
1*: Slight.
2: Little.
2*: Fair.
3: Good.
4: Excellent.

TABLE VII

Growth factor requirements of non-pathogenic (NP) and pathogenic (P) isolates of Piricularia oryzæ *(PO),* P. setariæ *(PS) and* Piricularia *sp. on* Eleusine coracana *(EC) with different carbon sources*

Treatments ..	Control						Thiamine						Biotin						Thiamine + Biotin						+ All					
Carbon source	PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
Xylose ..	1	–	–	0	–	–	1	–	1	0	2	1	1	1	–	0	–	–	2	3	2	4	4	4	2	3	2	4	4	4
Glucose ..	1	–	–	0	–	0	2	–	2	–	2	1	1	–	–	0	–	–	4	4	4	4	4	4	4	4	4	4	4	4
Galactose ..	1	–	–	–	–	0	2	–	1	–	2	1	1	1	–	–	–	–	2*	3	2*	3	2*	3	2*	3	2*	3	2*	3
Fructose ..	–	–	–	0	–	0	2	–	1	–	2	1	–	–	–	0	–	–	4	4	4	4	4	4	4	4	4	4	4	4
Sucrose ..	–	0	–	0	–	0	2	–	1	1	2	1	1	–	–	–	–	–	4	4	4	4	4	4	4	4	4	4	4	4
Lactose ..	1	–	–	0	–	–	1	–	1	–	2	1	1	–	–	1	–	–	2	2*	4	4	2*	3	2	2*	4	4	2*	3
Maltose ..	–	–	–	–	–	–	2	–	1	–	2	1	–	–	–	–	–	–	4	4	4	4	4	4	4	4	4	4	4	4
Mannitol .	0	0	–	–	–	0	0	0	–	–	1	–	0	0	–	–	1	0	0	0	–	–	1	–	0	0	–	–	1	–
Dulcitol ..	0	0	–	–	–	0	0	0	–	–	–	–	0	0	–	–	–	0	0	0	–	–	–	–	0	0	–	–	–	–
Glycerol ..	0	0	–	–	–	0	0	0	–	–	–	–	0	0	–	–	–	0	0	0	–	1	–	1	0	0	–	1	1	–

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

0: Nil.
–: Negligible.
1: Very slight.
2: Little.
2*: Fair.
3: Good.
4: Excellent.

and 10, a range of pH from 4·0 to 8·0 with unit intervals was chosen. pH was adjusted to the desired level with 6 N HCl or NaOH.

The data are presented in Table VIII.

Results.—Complete growth of the fungi occurred at all pH levels only when both thiamine and biotin were added to the medium. The presence of pyridoxine and inositol along with thiamine and biotin had no added benefit to the fungi at any of the pH levels tried. No marked differences were noted in growth of the fungi at the different pH levels. The results in general showed that pH of the medium did not alter the thiamine and biotin heterotrophy of the *Piricularia* spp., irrespective of their virulence.

12. *Nitrogen sources and vitamin deficiency of* Sclerotium oryzæ

The influence of different nitrogen sources on the vitamin deficiency of this fungus was studied on similar lines as Expt. 9.

The data are presented in Table IX.

Results.—Negligible or slight growth occurred in the controls with all the nitrogen sources. Thiamine alone promoted as much growth as a combination of the vitamins. Biotin, added singly, was without effect. This organism could not utilize ammonium tartrate. Nitrite was inhibitory. All other sources of nitrogen representing the inorganic nitrate and ammonia and organic nitrogen were utilized by this fungus. However, not all nitrogen sources were equally favourable for sclerotial production. While sodium nitrate, potassium nitrate, ammonium oxalate, urea and asparagine were very favourable for sclerotial production, ammonium nitrate, ammonium chloride and ammonium sulphate were not favourable. In general, the source of nitrogen did not alter the heterotrophy of this organism to thiamine.

13. *Carbon sources and vitamin deficiency of* Sclerotium oryzæ

Ten different sugars were tried in this study for their influence on the vitamin deficiency of this fungus. The procedure of Expt. 10 was followed.

The data are presented in Table X.

Results.—In the controls, where no vitamins were added, only negligible or slight growth occurred with all the carbon sources. Biotin alone did not promote growth. Thiamine when added singly or in conjunction with other vitamins promoted good growth of the fungus. No differences were noted between the different vitamin treatments, indicating thereby that the type of carbon source did not affect the thiamine heterotrophy of this organism.

TABLE VIII

Effect of pH on the vitamin deficiency of non-pathogenic (NP) and pathogenic (P) isolates of Piricularia oryzæ (*PO*), P. setariæ (*PS*) *and* Piricularia *sp. on* Eleusine coracana (*EC*)

Treatments	Control						Thiamine						Biotin					
pH	PO		PS		EC		PO		PS		EC		PO		PS		EC	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
4·0	0	0	0	0	0	0	1	2	1	2	2	2	–	–	–	0	–	0
5·0	0	0	–	–	0	0	2	2	2	2	2	2	–	–	–	–	–	–
6·0	–	0	–	–	0	0	4	2	2	2	2	2	–	–	–	–	–	–
7·0	0	0	–	–	0	0	2	2	2	2	2	2	–	1	–	–	–	–
8·0	0	0	–	0	0	0	2	2	2	2	2	2	1	–	–	–	–	–

Treatments	Thiamine + Biotin						+ All					
pH	PO		PS		EC		PO		PS		EC	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
4·0	4	4	4	4	4	4	4	4	4	4	4	4
5·0	4	4	4	4	4	4	4	4	4	4	4	4
6·0	4	4	4	4	4	4	5	4	4	4	4	4
7·0	4	4	4	4	4	4	4	4	4	4	4	4
8·0	4	4	4	4	4	4	4	4	4	4	4	4

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

0: Nil.
–: Negligible.
1: Very slight
2: Slight.
4: Good.
5: Excellent.

TABLE IX

Growth factor requirements of Sclerotium oryzæ *with different nitrogen sources*

Nitrogen source	Control	Thiamine	Biotin	Thiamine+ Biotin	+All	Sclerotial production in (+All)
Sodium nitrate ..	+	++++	+	++++	++++	Present
Potassium nitrate	+	++++	+	++++	++++	Present
Potassium nitrite	0	0	0	0	0	No growth
Ammonium nitrate	+	++++	+	++++	++++	Absent
Ammonium chloride	+	++++	+	++++	++++	Absent
Ammonium sulphate	+	++++	++	++++	++++	Absent
Ammonium oxalate	+	++++	−	++++	++++	Present
Ammonium tartrate	+	+	+	+	+	Absent
Urea ..	0	++++	−	++++	++++	Present
Asparagine ..	+	++++	+	++++	++++	Present

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

0: Nil. ++: Little.

−: Negligible. ++++: Good.

+: Slight.

Among the carbon sources tried, glucose, fructose and sucrose were better than xylose, galactose, lactose, maltose, mannitol and glycerol. No growth occurred with dulcitol. For sclerotial production, glucose, fructose, sucrose, lactose and maltose were more favourable than xylose, galactose, mannitol and glycerol.

14. *Growth of* Piricularia *spp. with different nitrogen sources in the presence of thiamine and biotin*

Sodium nitrate of the standard medium was replaced equivalently with 9 different nitrogen sources and the comparative ability of the different *Piricularia* spp. to utilize the various sources in the presence of 1·0 μg. of thiamine and 0·01 μg. of biotin are reported here. Cultures were grown in 250 ml. Erlenmeyer flasks (*vide* 'Methods').

TABLE X

Growth factor requirements of Sclerotium oryzæ *with different carbon sources*

Carbon source	Control	Thiamine	Biotin	Thiamine + Biotin	+ All	Sclerotial production in (+All)
Xylose ..	+	+++	+	+++	+++	Absent
Glucose ..	+	++++	+	++++	++++	Present
Galactose ..	+	+++	+	+++	+++	Absent
Fructose ..	+	++++	+	++++	++++	Present
Sucrose ..	+	++++	+	++++	++++	Present
Lactose ..	+	+++	+	+++	+++	Present
Maltose ..	+	+++	+	+++	+++	Present
Mannitol ..	+	++	+	++	++	Absent
Dulcitol ..	−	−	−	−	−	Absent
Glycerol ..	+	+++	+	+++	+++	Absent

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

−: Negligible. +++: Fair.
+: Slight. ++++: Good.
++: Moderate.

The data are presented in Table XI.

Results.—All *Piricularia* spp. could utilize organic amide or amino nitrogen and inorganic nitrate nitrogen. They could not, however, utilize inorganic ammonia nitrogen, as in ammonium nitrate, ammonium chloride and ammonium sulphate. Potassium nitrite was without exception toxic. In general, asparagine, ammonium tartrate, potassium nitrate, sodium nitrate and ammonium oxalate were better sources of nitrogen than the rest.

15. *Growth of* Piricularia *spp. with different carbon sources in the presence of thiamine and biotin*

Comparative studies on the ability of different *Piricularia* spp. to utilize various carbon sources in the presence of 1·0 μg. thiamine and 0·01 μg. biotin are reported here.

TABLE XI

Growth of Piricularia *spp. with different nitrogen sources in the presence of thiamine and biotin*

Nitrogen source	Mat weight (mg.)					
				Isolates from		
	P. oryzæ A	*P. setariæ* B	*P. zingiberi* C	*E. coracana* D	*T. vulgare* E	*B. mutica* F
1 Sodium nitrate	25·4	30·2	46·5	42·7	23·5	46·7
2 Potassium nitrate	34·1	33·1	41·7	38·4	24·1	48·8
3 Potassium nitrite	0·0	0·0	0·0	0·0	0·0	0·0
4 Ammonium nitrate	7·0	11·3	3·3	7·2	12·7	10·8
5 Ammonium chloride	4·1	7·0	2·9	3·6	7·1	7·4
6 Ammonium sulphate	3·3	6·8	3·1	4·7	6·5	8·4
7 Ammonium oxalate	27·5	30·3	4·2	35·7	27·5	53·3
8 Ammonium tartrate	30·7	43·2	24·0	31·7	34·5	52·9
9 Urea	31·1	34·9	24·9	37·2	27·7	41·5
10 Asparagine	42·5	47·5	48·7	85·0	46·7	46·7
Significance by F. test	Yes	Yes	Yes	Yes	Yes	Yes
C.D. at 1% level	2·1	6·0	4·9	4·2	4·4	4·6

Conclusions

A: 10 2 9 8 7 1 4 5 6 3

B: 10 8 9 2 7 1 4 5 6 3

C: 10 1 2 9 8 7 4 6 5 3

D: 1 2 9 7 10 8 4 6 5 3

E: 10 8 9 7 2 1 4 5 6 3

F: 7 8 2 1 10 9 4 6 5 3

Sucrose of the standard medium was replaced with 10 different carbon sources and the data are presented in Table XII.

TABLE XII

Growth of Piricularia *spp. with different carbon sources in the presence of thiamine and biotin*

Carbon source	Mat weight (mg.)					
				Isolates from		
	P. oryzæ A	*P. sataria* B	*P. zingiberi* C	*E. coracana* D	*T. vulgare* E	*B. mutica* F
1 Xylose	25·1	35·8	15·7	7·9	17·9	35·7
2 Glucose	28·9	21·1	43·0	41·2	19·5	37·3
3 Galactose	2·5	14·2	4·6	2·5	17·8	4·8
4 Mannose	25·1	19·3	35·4	32·2	18·2	35·2
5 Fructose	28·8	21·7	30·9	38·3	17·3	34·1
6 Sucrose	32·3	23·2	40·1	34·3	16·9	41·4
7 Lactose	23·3	31·4	25·7	6·2	20·5	27·9
8 Maltose	20·9	16·8	9·1	4·3	17·0	41·5
9 Mannitol	4·9	3·1	1·1	1·1	7·8	38·3
10 Dulcitol	1·1	1·8	5·3	0·3	3·1	16·2
11 Glycerol	2·0	0·0	0·0	0·2	0·0	6·8
Significance by F. test	Yes	Yes	Yes	Yes	Yes	Yes
C.D. at 1% level ..	5·1	7·0	8·1	9·2	2·5	4·4

Conclusions

A: 6 2 5 1 4 7 8 9 3 11 10

B: 1 7 6 5 2 4 8 3 9 10 11

C: 2 6 4 5 7 1 8 10 3 9 11

D: 2 5 6 4 1 7 8 3 9 10 11

E: 7 2 4 1 3 5 8 6 9 10 11

F: 8 6 9 2 1 4 5 7 10 11 3

Results.—The utilization of the different carbon sources varied with the type of isolate. They were: *Piricularia oryzæ*: Sucrose, glucose and fructose were the best sources of carbon. Higher alcohols and galactose were poor sources.

P. setariæ: Xylose and lactose were the best sources. This isolate could, however, utilize galactose to a certain extent. Higher alcohols were poorly utilized.

P. zingiberi: Glucose, sucrose and mannose were the best. Maltose, galactose and the higher alcohols proved to be poor sources of carbon.

Piricularia sp. from *Eleusine coracana*: Glucose, frcutose, sucrose and mannose were the best sources. All others were definitely inferior.

Piricularia sp. from *Triticum vulgare*: Lactose was the best source of carbon compared to xylose, galactose, fructose and maltose. Glucose and mannose were as good as lactose. Higher alcohols were poor sources.

Piricularia sp. from *Brachiaria mutica*: Maltose and sucrose were the best compared to xylose, mannose and fructose. Among the higher alcohols, mannitol and to a certain extent dulcitol were used by this isolate. Glycerol and galactose were poorly utilized.

Discussion

While a number of filamentous fungi have been reported for their vitamin deficiencies (Robbins and Kavanagh, 1942), admittedly, quite a few of them have been investigated intensively with regard to several important factors that are known to influence the vitamin heterotrophy of a fungus. The present work, wherein attention has been particularly paid to such limiting factors as the carry over of growth factors with the inoculum, the influence of the source of nitrogen and carbon, pH of the medium and behaviour of different strains of the same fungus, is discussed in the light of some general concepts derived from the accumulated knowledge on vitamins and growth factors for fungi.

Robbins and Kavanagh (1942) recognized two kinds of heterotrophy, *viz.*, complete and partial. The former is termed by Lilly and Barnett (1951) as total. Both complete and partial deficiencies may be single, for one vitamin or multiple, for more than one vitamin. The widely worked out fungus *Phycomyces blakesleeanus* is a classical example of complete deficiency for a single vitamin, thiamine (Schopfer, 1943). In the case of *Lenzites trabea* (Lilly and Barnett, 1948) the deficiency is partial since the rate of growth of this organism doubled in the presence of thiamine, while

in its absence, the fungus still attained maximum growth, though slowly. *Sordaria fimicola* for biotin (Lilly and Barnett, 1947) and *Phytophthora* spp. for thiamine (Robbins, 1938) may be cited as other examples of fungi deficient for a single vitamin. *Ceratostomella pini* (Robbins and Ma, 1942 *b*), *Ophiobolus graminis* (White, 1941), *Melanospora destruens* (Hawker, 1939 *a*) and *Lambertella pruni* (Lilly and Barnett, 1951) are some of the fungi reported to be deficient for thiamine and biotin, illustrating multiple deficiencies. Such deficiencies may again be total or partial. *Ophiobolus graminis* has been found to be highly or completely deficient for both thiamine and biotin (White, 1943). The individual vitamins themselves had no effect on this fungus. On the contrary, thiamine or biotin alone could promote some growth of *Lambertella pruni* indicating a partial deficiency of this organism to the respective vitamins (Lilly and Barnett, 1951). *Balstocladia pringsheimii* (Cantino, 1948) and *Ceratostomella ips* no. 255 (Robbins and Ma, 1942 *a*) are fungi showing deficiencies for more than two vitamins.

On the basis of the above classification, the *prima facie* evidence offered by the results of Expts. 1 and 2 indicates that all the species and isolates of *Piricularia* investigated show a deficiency for both thiamine and biotin (Tables I and II). *Sclerotium oryzæ*, on the other hand, has but a single deficiency for thiamine (Table III and Plate XXI).

Examination of Table I indicates that, while omission of thiamine from the vitamin mixture reduced growth of the different isolates by 62-84%, withdrawal of biotin brought down growth only by 10 to 29% except in the case of *Piricularia* sp. on *Eleusine coracana* where this reduction amounted to 46%. This naturally leads to the *a priori* assumption that the isolates of *Piricularia* are highly deficient or near totally so for thiamine and partially for biotin. However, none of these isolates were able to grow with thiamine or biotin, added singly to the medium (Table II). Complete growth resulted only in the presence of a combination of both vitamins showing thereby that the fungi in question are heterotrophic to thiamine as well as biotin.

The discrepancy in the results between the two experiments may be explained as being due to the different methods of inoculation used. In Expt. 1 a pin-head size of aerial mycelium without any adhering agar was used as inoculum after Robbins and Ma (1945). But, a loopful of mycelial suspension (*vide* 'Methods') formed the inoculum in Expt. 2. The very fact that growth amounting to 3 to 19% occurred in the controls in Expt. 1 is suggestive of the carry over of some quanta of the vitamins with the inoculum. It is not, therefore, surprising that biotin, carried over with the

inoculum, perhaps in sub-optimal doses, has, nevertheless, contributed to appreciable growth of the fungi in the biotin-omitted treatments, in view of its activity in extremely minute quantities.

Further proof of the totality of the deficiencies of the species and isolates of *Piricularia* to thiamine as well as biotin and of *Sclerotium oryzæ* to thiamine is offered by a consideration of the response of these fungi to different levels of the vitamins over a sufficiently long period of incubation facilitating maximum growth (Text-Figs. 1–7). Based on the results of such a response the following generalizations can be made:

1. Growth of all the seven fungi is negligible in the absence of the vitamins in the medium. 2. Positive effect of the vitamins is felt only when they are present in amounts excess of 0·01 μg. per flask. 3. Significant growth is promoted by 0·1, 1·0, 10·0 μg. levels of vitamins and with these levels maximum growth is reached on the 12th day in the case of *P. oryzæ*, *P. setariæ*, *P. zingiberi*, *Piricularia* sp. from *Brachiaria mutica* and *Sclerotium oryzæ* and on the 9th day by *Piricularia* spp. from *Eleusine coracana* and *Triticum vulgare*. The fact that none of these fungi is able to make any appreciable growth even after prolonged incubation is indicative of the totality of their deficiencies. However, this total deficiency is multiple (for thiamine and biotin) for *Piricularia* spp. and single (for thiamine only) for *S. oryzæ*.

The optimum levels of the vitamins required by these fungi are found to be 1·0 μg. of thiamine and 0·01 μg. of biotin for all the species and isolates of *Piricularia* and 1·0 μg. of thiamine for *S. oryzæ* (Table IV and Text-Fig. 8). Even though the six isolates of *Piricularia* consisted of three distinct species and three isolates from different hosts, they did not exhibit any qualitative or quantitative differences in their vitamin requirements under the cultural conditions of the investigation.

The vitamin deficiencies of *Piricularia oryzæ*, *P. setariæ*, *Piricularia* sp. on *Eleusine coracana* and *Sclerotium oryzæ* are further considered with reference to the influence of the cultural environment. It must be emphasized that much attention has not been paid in this regard with many of the fungi reported to be vitamin-deficient. However, on the available evidence, Robbins and Kavanagh (1942) recognized two classes of vitamin deficiencies, *viz.*, absolute and conditioned. The total deficiency of *Phycomyces blakesleeanus* (Hawker, 1950; Lilly and Barnett, 1951) and *Ceratostomella fimbriata* (Lilly and Barnett, 1951) to thiamine has not been known to be altered by any environmental conditions. The deficiency of such fungi is termed absolute whereas in other cases the vitamin requirement of

a fungus is largely dependent on the cultural environment. The influence of the source of nitrogen is reported with a mutant of *Neurospora sitophila* (Stokes *et al.*, 1943) where the fungus is found to require pyridoxine with nitrate, amino or amide sources of nitrogen. But, the need for an external supply of the vitamin is obviated in the presence of ammonium salts. The different *Piricularia* spp. and *Sclerotium oryzæ*, on the contrary, need only thiamine and biotin and thiamine respectively with inorganic nitrate, organic ammonium and amide sources of nitrogen. Pyridoxine or inositol is neither essential nor stimulatory with any of the nitrogen sources tried (Tables VI and IX). The red yeast *Rhodotorula sanniei* may be quoted as an example of the influence of the source of carbon on the vitamin requirement of a fungus. This organism requires thiamine when glucose formed the carbon source. But, when glycerol replaced glucose, thiamine is not needed (Fromageot and Tschang, 1938). Investigations in this direction on the *Piricularia* spp. and *Sclerotium oryzæ* have shown that replacing sucrose of the standard medium with nine different sugars does not alter the heterotrophy of these fungi, *i.e.*, thiamine and biotin for *Piricularia* spp. and thiamine to *S. oryzæ* (Tables VII and X). Further, the results indicate that the *Piricularia* spp. used in these experiments do not grow with glycerol. Even a combination of thiamine, biotin, pyridoxine and inositol is without effect with this source of carbon. On the other hand, *S. oryzæ* which is able to grow with glycerol still needs thiamine (Table X). Yet another such instance is the pH of the culture medium influencing the vitamin deficiency of a fungus and is illustrated by *Sordaria fimicola* (Lilly and Barnett, 1947). While partial thiamine deficiency of this fungus is noted at the pH range of 3·4 to 3·8, no such deficiency is apparent at pH 4·0 and above. This, however, is not the case with *Piricularia* spp. A range of pH from 4·0 to 8·0 does not alter the thiamine and biotin heterotrophy of these fungi (Table VIII).

Thus, in view of the consistent heterotrophy of these fungi to thiamine and biotin (*Piricularia* spp.) and thiamine (*S. oryzæ*), regardless of the source of nitrogen, carbon and pH of the culture medium, they may be considered to have an aboslute and total deficiency for the respective vitamins.

In the above discussion, the vitamin deficiencies of *Piricularia* spp. have been comparatively dealt with. Speaking of *P. oryzæ*, in particular, the results confirm its total multiple deficiency to thiamine and biotin. This result, while in conformity with that obtained by Leaver *et al.* (1947), differed in the following respects with the later work in Japan. Otani (1952) found that biotin is essential for this organism with thiamine as a complemental factor. Tanaka and Katsuki (1953) confirmed the findings of Otani

(*loc. cit.*) and stated that thiamine was only stimulatory. Such divergent results in the vitamin deficiencies of one and the same species of a fungus are not uncommon. While certain strains of *Boletus granulatus* had an absolute demand for thiamine, others were only strongly stimulated by this vitamin (Melin and Nyman, 1941). Similar observations were made on *Marasmius perforans* (Lindeberg, 1944) and *Ophiostoma ulmi* (Fries, 1943).

The study of the vitamin requirements of *Piricularia* spp. was further extended to a consideration of the vitamin needs of non-pathogenic and pathogenic isolates. But the pathogenic strains of *P. oryzæ*, *P. setariæ* and *Piricularia* sp. from *Eleusine coracana* scarcely differed from the non-pathogenic strains in their heterotrophy to thiamine and biotin, quite independent of the source of nitrogen, carbon or pH of the medium (Tables VI–VIII). That vitamin deficiencies have no corrleation with loss in pathogenicity has been similarly reported in the case of *Venturia inaequalis* (Leben and Keitt, 1948).

Mathew (1954) observed that the fungus *Pellicularia koleroga* responded to different vitamins equally well when the isolate was fresh but ultimately became heterotrophic only to thiamine on repeated sub-culturings on artificial media. Such a phenomenon was not noticed with any of the *Piricularia* spp. tried. The fresh isolates maintained on 2% potato-dextrose agar for well over three years did not show any difference in their heterotrophy, with time. Further, that the substrate of the stock culture has no bearing on the vitamin heterotrophy has been shown (Expt. 8 and Table V).

Even though all *Piricularia* spp. used in this work are uniform in their heterotrophy to thiamine and biotin, their physiological abilities to use various nitrogen and carbon compounds varied somewhat with the type of the isolate (Tables XI and XII). But, some broad generalizations can be made which are considered below incidentally with the physiology of *S. oryzæ*. While organic ammonium and amide nitrogen as well as inorganic nitrate nitrogen were assimilable by all the *Piricularia* spp. inorganic ammonium sources of nitrogen proved non-assimilable under the cultural conditions of the investigation. This was found not to be due to a conditioned deficiency of pyridoxine or inositol (Table VI).

Sclerotium oryzæ, on the other hand, utilized all the three forms of nitrogen though sclerotial production was sparse with inorganic ammonium sources of nitrogen (Table IX). These results do not agree with those obtained by Tomizowa (1953) with *P. oryzæ* where this fungus has been reported to grow profusely with ammonium sulphate. But this inability

to use inorganic ammonium nitrogen has been likewise noted with *Metarrhizium glutinosum* (Brian *et al.*, 1947).

Foster's (1949) stand that the inability of an organism to utilize various forms of nitrogen must be re-examined in the light of its growth factor requirements is very well brought out by this work on different *Piricularia* spp. Working on *P. oryzæ*, *P. setariæ* and *Piricularia* sp. on *Eleusine coracana*, Ramakrishnan (1948) obtained very poor growth of these fungi on KNO_3 but comparatively better growth with asparagine, urea and peptone. However, in this work, mention is not made regarding the purity of chemicals or addition of vitamins to the medium. Since the present work has shown the ability of all *Piricularia* spp. to use both potassium nitrate and urea equally well, the poor growth of these fungi with potassium nitrate in the previous case is evidently due to the lack of an external supply of thiamine and biotin. The better growth observed with asparagine, urea and peptone can be explained on the basis of the presence of growth factors in these chemicals. That commercial asparagine carries some biotin as contaminant has been reported (Robbins and Schmidt, 1939).

All the *Piricularia* spp. and *Sclerotium oryzæ* were found to grow with a variety of carbon sources, though their relative capacities to utilize the different sugars somewhat varied (Tables XII and X). Higher alcohols, in general, proved to be poor sources of carbon. Similar observations were made on *P. oryzæ* by Otani (1953) and Tomizowa (1953).

As to the possibility of using the fungi investigated for bioassays, these may not be preferred despite their consistent heterotrophy in view of the availability of several mutants of *Neurospora* (Beadle and Tatum, 1945) which by virtue of their rapid growth rate facilitate quick assays.

Finally, the present study may be examined on its possible significance in the taxonomy of *Piricularia* and host-parasite relationships.

Apart from the four species of *Piricularia* recognized by Padwick (1950), the taxonomic position of other isolates of *Piricularia* infecting various hosts is still uncertain. The present work has revealed the uniformity of the different species and isolates of *Piricularia* in their heterotrophy to thiamine and biotin. This naturally tends to reduce the importance of this study in the taxonomy of *Piricularia*. Nevertheless, further studies on the response of these fungi to different substitution products of thiamine and biotin might aid in fixing up the taxonomic position of *Piricularia* spp. infecting *Eleusine coracana*, *Triticum vulgare*, *Brachiaria mutica* and a number of other graminaceous plants.

The probable significance of the growth factor requirements of fungi in relation to the presence of growth substances in host cells has been discussed by Gäumann (1950) and Allen (1954). Work in this laboratory (Subramanyan and Suryanarayanan, unpublished) has indicated that desthiobiotin not only does not replace normal biotin for *P. oryzæ* but also inhibits growth. Speculatory as yet, it is not unlikely that such unsuitable analogues are present in the resistant types especially on the leaf surface as products of exosmosis and their vital role at the " infection-court " in the pre-penetration stages can hardly be ruled out.

Summary

Detailed studies made on *Piricularia oryzæ*, *P. setariæ*, *P. zingiberi*, *Piricularia* spp. on *Eleusine coracana*, *Triticum vulgare* and *Brachiaria mutica* and *Sclerotium oryzæ* showed them to have a total heterotrophy to vitamins which seemed to be absolute. All the species and isolates of *Piricularia* had a multiple deficiency to thiamine and biotin while *S. oryzæ* had a single deficiency to thiamine.

The optimal amounts of vitamins for these fungi were found to be about 1·0 μg. of thiamine and 0·01 μg. biotin per flask in the case of *Piricularia* spp. and 1·0 μg. thiamine for *S. oryzæ* under the cultural conditions of investigation.

The heterotrophy of these fungi to the respective vitamins was not altered by the type of nitrogen or carbon source and at various pH ranges, thereby indicating their vitamin deficiencies to be absolute and not conditioned by the cultural environment. No correlation was found to exist between the vitamin requirements of these fungi and their virulence.

The physiology of these fungi, considered from the aspect of their growth factor requirements, revealed the inability of *Piricularia* spp. to utilize inorganic ammonium sources of nitrogen. *S. oryzæ*, on the other hand, utilized inorganic nitrate, ammonium nitrogen and organic nitrogen. Nonetheless, inorganic ammonium sources of nitrogen were not favourable for sclerotial production by this fungus. The unsuitability of this form of nitrogen to the growth of *Piricularia* spp. and sclerotial formation by *S. oryzæ* was found to be not due to a conditioned deficiency of pyridoxine or inositol.

The fungi investigated were found to use a variety of carbon sources. In general, sugars were better sources of carbon than higher alcohols. The importance of the study of growth factors in fungal physiology is discussed.

The utility of this study in the taxonomy of *Piricularia*, the usefulness of the organisms for bioassays and the probable significance of this

investigation in understanding disease resistance to *Piricularia* are discussed in a general way.

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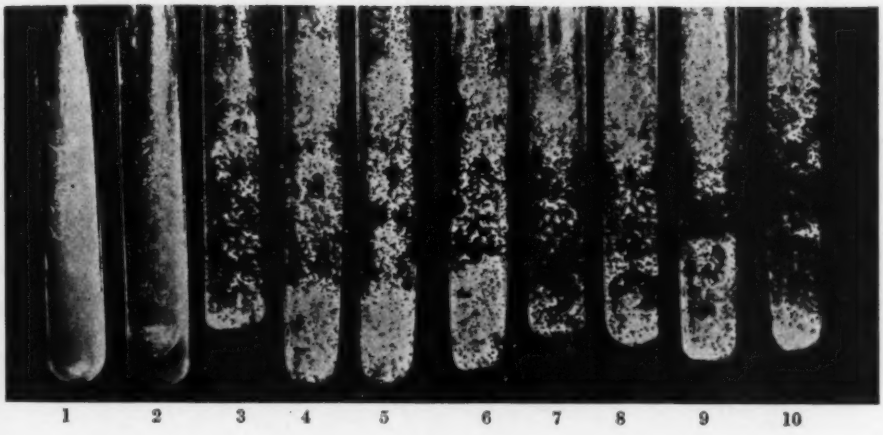

Plate showing the effect of excluding a single vitamin from a vitamin mixture (+All) on growth of *Sclerotium oryzæ*.

1. Control. 2. Thiamine. 3. Biotin. 4. Pyridoxine. 5. Nicotinic acid. 6. Inositol. 7. Pantothenic acid. 8. *p*-Amino benzoic acid. 9. Riboflavin. 10. + All vitamins.

QUANTITATIVE STUDY OF PLANKTON OFF LAWSON'S BAY, WALTAIR

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Introduction

The significance of plankton in the productivity of the seas is too well known and it is superfluous to quote here the extensive literature on the subject. In assessing the productivity, exact quantitative methods have to be employed and the observations carried out round the year. The available literature on marine productivity in tropical seas is very meagre and this is particularly true of the seas around India. The existing accounts are mainly of a qualitative nature with some information on the relative abundance of some groups of planktonic organisms during the different months of the year. It is a well recognised fact that the prevailing hydrographical conditions in any zone have a direct bearing on phytoplankton production. Previous workers from this Department have shown (Ganapati and Murthy,

1953, 1955; Ganapati and Rao, V. R., 1954; Ganapati and Rao, T. S. S., 1954) that there is a direct correlation between the abundance of some planktonic organisms and the prevailing hydrographical conditions off this coast.

The present investigation was carried out to assess the plankton production at a fixed station in the 10 fathom line off Lawson's Bay, Waltair.

MATERIAL AND METHODS

The plankton samples were collected from January to December, 1956, twice a week, between 6 and 7 A.M. A known volume of water was filtered through a fine bolting silk net of mesh size No. 30. The concentrated sample was transferred to a glass container wrapped in moist linen and the organisms examined in the living condition in the laboratory. Four such samples were taken till March and an additional fifth sample for the remaining months. Owing to the rough weather conditions the quantitative estimations for the month of May could not be worked out. Quantitative estimations were made as detailed below.

1. *Numerical estimation.*—After a preliminary examination in the living condition the sample was fixed by the addition of sufficient quantity of 40% formalin to make the total strength of dilution of the sample to about 5%. The technique of diluting and counting suggested by Nielsen (1933) was adopted and numerical estimations made by using Utermohl's inverted plankton microscope. The number of organisms in one litre of water was calculated. The macroplankton organisms were separated before counting.

2. *Volumetric estimation.*—The fixed sample was poured into a special graduated conical sedimentation funnel and the organisms allowed to settle for at least 24 hours and the volume of plankton noted.

3. *Pigment extraction.*—Harvey's method of pigment extraction (1934) was adopted using three different solvents: Acetone; Ethyl alcohol and Benzene; and Methyl alcohol. The concentration of the pigment was estimated by using a Lumetron Photoelectric Colorimeter with 420 μ filter.

4. *Total biomass.*—Bogorov's method (1934) of total Biomass estimation was employed. The fixed plankton sample was filtered through a weighed Whatman No. 42 filter-paper disc of 4·5 cm. diameter. The sample was washed with distilled water thrice to free the salt content. The total Biomass was now estimated by taking the constant dry weight of the plankton and expressed as mg./M^3 of sea-water.

5. *Total organic matter.*—Total organic matter was determined, as suggested by Riley (1938 and 1941) and Verudin (1951) by incineration of

the dried plankton and the difference between the constant dry weight, *i.e.*, total Biomass and the ash gives the total organic matter oxidised. The values are expressed as mg./M³ of sea-water. All the weighings were made in the Metler semi-microelectric balance.

6. *Hydrography.*—Surface temperature was measured with a bulb thermometer of 0·2° C. calibration by dipping the thermometer in the water directly from the Catamaran.

Salinity:	Knudsen's method
Oxygen:	Winkler's method.
Inorganic phosphates	Denige's method as modified by Robinson and Thompson (1948).
Silicates:	Dienert and Wandenbulcke method as modified by Robinson (1948).

TABLE I

Table showing the hydrographical conditions for the year 1956

Months	Rainfall in inches	Temperature ° C.	Salinity ‰	Dissolved oxygen ml,/1.	Phosphates μg. at/1.	Silicates μg. at/1.
January ..	0·10	24·92	28·82	3·68	1·37	7·05
February ..	0·0	25·85	33·08	3·61	0·41	11·68
March ..	0·0	25·79	34·37	5·02	0·79	9·43
April ..	0·95	25·40	34·44	3·87	0·78	10·86
May ..	2·71	27·91	33·75	3·22	0·52	13·62
June ..	4·39	28·12	33·33	1·66	0·82	12·68
July ..	9·05	28·87	31·26	3·20	0·69	11·62
August ..	5·20	28·46	32·15	3·28	0·52	11·08
September ..	11·09	29·50	31·33	3·79	0·53	11·73
October ..	20·05	28·95	17·40	4·16	0·82	29·91
November ..	0·09	27·04	23·95	3·60	0·81	21·84
December ..	0·0	25·04	26·26	4·66	0·70	16·84

HYDROGRAPHICAL CONDITIONS

A series of papers (Ganapati and Murthy, 1954, 1955; Ganapati, La Fond and Bhavanarayana, 1956; La Fond, 1954, 1957; La Fond and Bhavanarayana, 1957; Bhavanarayana and La Fond, 1957; Ganapati and Subba Rao, 1957; Poornachandra Rao, 1957 and Rama Sastry and Balararamamurty, 1957) on the hydrographical conditions of the Bay of Bengal off the east coast of India with particular concentration off Waltair have appeared from the Andhra University and we have now a fairly accurate picture of the prevailing hydrographical conditions and the water movements during the different months of the year. The data collected during the year 1956 have, in the main, substantiated conditions reported for the previous years and the salient features are recorded below. The monthly average values of temperature, salinity, dissolved oxygen, phosphates and silicates are given in Table I and Fig. 1.

Temperature.—A high range of temperature was observed during the year, a maximum of 30·2° C. on 5–9–1956 and a minimum of 24·4° C. on 19–12–1956. A monthly average maximum of 29·5° C. during September and a minimum of 25·04° C. during December were observed. The low temperatures observed during March and April have been shown as due to upwelling (Ganapati and Subba Rao, 1957). The high temperatures during September and October are due to the sinking phenomenon of the surface waters (La Fond, 1954).

Salinity.—A high range of salinity was observed with a maximum of 34·88‰ on 4–4–1956 and a minimum of 13·57‰ on 8–10–1956. A monthly average maximum of 34·44‰ during April and a minimum of 17·40‰ during October were observed. The salinity of the waters is mainly influenced by the currents. The high salinity values during the northerly current period, January to June, are mainly due to incursion of the oceanic waters from the central Indian Ocean (Sewell, 1929); diminution of the flow of the rivers to the south (Sewell, 1929); and upwelling of the highly saline bottom waters (Ganapati *et al.*, 1956 and Ganapati and Subba Rao, 1957). The lowest values during October to November period are due to the dilution by large volumes of fresh-water brought by the southerly current from rivers to the north (Sewell, 1929). An inverse relationship between the salinity and silicates was observed all through the year.

Dissolved oxygen.—The dissolved oxygen content ranged from 5·67 ml./l. on 9–3–1956 to 0·33 ml./l. on 7–6–1956. The monthly average values ranged from 5·02 ml./l. during March to 1·66 ml./l. during June. A direct relationship between the phytoplankton and dissolved oxygen and

an inverse relationship between temperature and dissolved oxygen during the period January to June were observed.

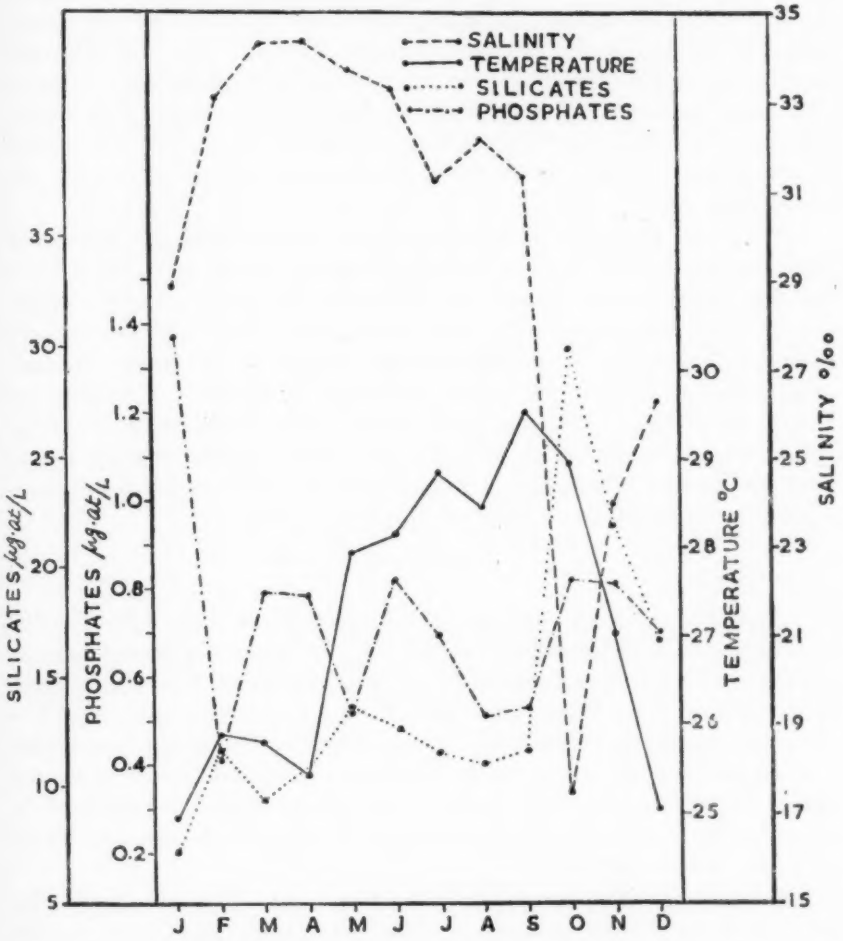

FIG. 1. Showing the mean monthly values for temperature, salinity, silicates and phosphates in the surface waters off Lawson's Bay during the year 1956.

Phosphates.—The phosphate values ranged from 1·8 on 12–1–1956 to 0·32 µg. at./1. on 28–2–1956. The monthly average values ranged between 1·37 and 0·41 µg. at./1. during January and February respectively. Sewell (1932) observes that during January one branch of the bottom Antarctic

drift, " Bends towards the north and flows up into the Bay of Bengal along the east coast of India " and this nutrient-rich Antarctic drift may account for the high phosphate value in January. The high phosphate values during October to November may be due to the large quantities of river borne nutrients brought by the southerly current. The low value for February may be due to the rapid utilisation of the phosphates in the surface waters by the spring outburst of phytoplankton which had its major peak during March. The sudden rise in phosphates during the March to April periods is due to the upwelling of nutrient-laden bottom waters (Ganapati and Subba Rao, 1957).

Silicates.—A high range of silicates was observed with a maximum of 39·71 on 21–10–1956 and a minimum of 5·0 μg. at./l. on 9–1–1956. The monthly average values ranged from 29·91 to 7·05 μg. at./l. during October and January respectively. The fairly high values during March to April periods in spite of the major phytoplankton bloom are due to the upwelling of the bottom nutrient-rich waters (Ganapati, *et al.* 1956; Ganapati and Subba Rao, 1957). The maximum values during October to November period may be attributed to the influx of the river waters brought by the southerly current. The influx of fresh-water is also indicated by considerable lowering of the salinity during this period.

PLANKTON

(*a*) *Numerical*

Phytoplankton.—The phytoplankton cell numbers vary from 25405/l. in March to 94/l. in December (Table II, Fig. 2). There is a gradual increase in their numbers from January to March when the primary peak is reached. From April onwards there is a steep fall until July. In August there is a slight increase which becomes pronounced from September onwards leading to a secondary peak in October to November. This is followed by a steep fall in December. The main bulk of the phytoplankton is constituted by the diatoms, the dinoflagellates occurring only in small numbers during both the bloom periods.

Zooplankton.—The zooplankton shows a major peak in March coinciding with the phytoplankton peak. There is a rapid fall in their numbers from April onwards with a small increase during August and November. The total zooplankton numbers varied from 414/l. to 16/l. during March and December respectively as given in Table II and Fig. 5.

(*b*) *Volume*

A high range of plankton volume was observed during the period of study with the maximum value of 375·0 c.c./M^3 of sea-water on 25–10–1956

TABLE II

The planktological conditions for the year 1956

Months	Pl. Vol. c.c./M³	Phy. pl. orgs. Nos./l		Zoopl. orgs. Nos./l	Pigment units/M³ of S.W.			Total Bio. mg./M³.	Total org. mat. mg./M³
		Diatoms	Dinofl.		Acetone	Alco-Benz.	Meth. Alco.		
January ..	55·94	1273	71	68	736·619	580·535	..	123	28
February ..	63·12	3084	177	239	1587·5	1738·437	..	1230	712
March ..	107·81	24432	973	414	7722·5	9752·656	8834·83	1171	664
April ..	89·37	3915	471	198	8425·625	8325·0	8125·0	432	144
May ..	..	..	..	..	1212·5	..	..	..	..
June ..	26·25	1986	166	164	1335·0	2037·19	1508·125	534	467
July ..	9·16	166	108	144	412·708	211·41	407·916	252	144
August ..	6·46	1023	100	169	153·83	196·409	193·229	131	45
September ..	6·25	881	101	118	119·322	154·718	121·987	559	164
October ..	194·16	3980	116	60	1882·291	1517·875	1573·666	359	132
November ..	129·99	3724	88	70	665·818	808·118	765·416	966	595
December ..	14·55	80	14	16	66·5	107·209	104·721	402	77

and a minimum of 2·5 c.c./M³ of sea-water on 7th June, 18th August and 13th September 1956. The monthly average values ranged from 194·16 c.c./M³ to 6·25 c.c./M³ of sea-water during October and September respectively. Fairly high values of plankton volumes were observed during March, April and November. The volumetric values of plankton closely followed and ran parallel with those obtained by other methods of estimation.

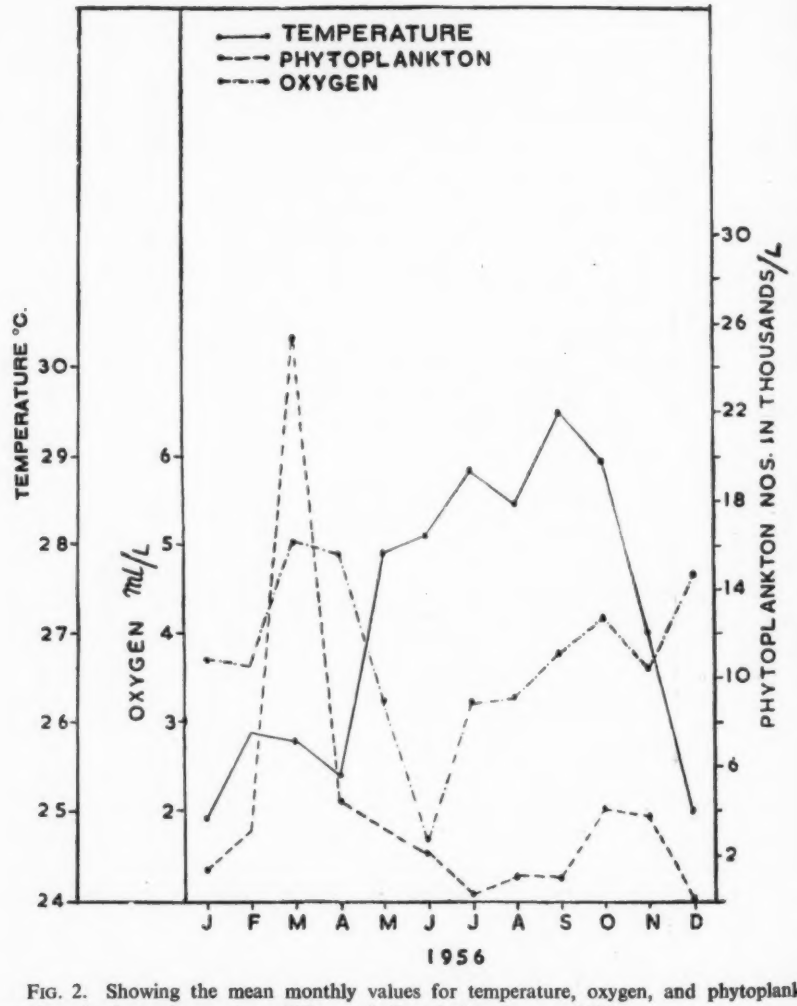

FIG. 2. Showing the mean monthly values for temperature, oxygen, and phytoplankton organisms in the surface waters off Lawson's Bay during the year 1956.

During periods of rich plankton volume the phytoplankton was present in appreciable quantities. However, the higher values recorded during October and November are largely due to the occurrence of big medusoids like *Aequorea* in the collections. From January onwards the plankton volume gradually increased, up to March, followed by a steep fall after April. The total plankton is poor from May to September with a slight increase in June.

(*c*) *Pigments*

Riley (1941) made a comparative study of the various methods of estimation of plankton like numerical, volumetric, gravimetric and finally pigment extraction also. He calculated the correlation between the different methods and found that the pigment extraction is more accurate than other methods, to know the synthetic potentialities of the sea. Tucker (1949) observed that this method had the advantage of being simple and more rapid than any other method of estimation and he correlated the counted numbers of the plankton and the density of the pigment extraction. Gardiner (1943) suggested that acetone cannot extract all the chlorophyll pigments from certain phytoplankton organisms like *Ceratium* and some other dinoflagellates. He recommended alcohol-benzene as a better solvent than acetone. So far there are only two works on chlorophyll estimations of phytoplankton from Indian waters. The first is that of Thompson and Gilson (1937) during the "John Murray Expedition", for a few stations in the Indian Ocean, and the other that of Ramamurthy (1953 *c*) off Madras. The values are relative as they are based on townet collections. In the present work acetone and ethyl alcohol-benzene were used as solvents throughout and in addition methyl alcohol (in hot) from March onwards. As suggested by Graham (1943 *a*) the solvent was added every time just sufficient to extract the pigments. The values are shown in Table II.

A high range of pigment units with acetone as the solvent was seen. The maximum of 18550 H.U./M^3 and a minimum of 27·5 H.U./M^3 were seen on 16th March and 19th December respectively. The monthly average values ranged between 8425·625 H.U./M^3 and 66·5 H.U./M^3 during April and December respectively.

With ethyl alcohol-benzene a maximum of 27050 H.U./M^3 on 9th March and a minimum of 28·125 H.U./M^3 on 5th January were observed. The monthly average values ranged from 9752·656 H.U./M^3 to 107·209 H.U./M^3 during March and December respectively. Fairly high values have been observed during April also.

Pigment units with methyl alcohol as the solvent ranged from 24192 H.U./M^3 to 55 H.U./M^3 on 9th March and 9th December respectively. The monthly average values ranged between 8834·83 H.U./M^3 and 104·721 H.U./M^3 during March and December respectively.

In general the pigment extraction method ran parallel with the plankton production as estimated by other methods. From January onwards there is a gradual increase up to March–April periods when the major peak of phytoplankton production was observed. There is a gradual fall from June up to September. A minor peak was observed during October to November period following which a steep fall was seen during December when the minimum value was observed.

It is interesting to note that the mean monthly maxima of the pigment units with different solvents differed. With acetone the maximum values are in April, while with alcohol-benzene and methyl alcohol it is during March. The monthly minimal values agree to fall during December with all the three solvents.

It is interesting again to note that the pigment values with alcohol-benzene from February to June are higher than those obtained with either acetone or methyl alcohol. During the same period the dinoflagellates were more abundant than in the other months and it is possible that the higher values with alcohol-benzene may be due to the capacity of this solvent to extract the pigments more completely from the dinoflagellates than the other two solvents (Table II).

In general the cell count and the pigment values parallelled each other during the period of study except during the summer months when the pigments were significantly higher than the cell counts. Conover (1956) and Riley (1952) also observed a discrepancy between the cell numbers and the chlorophyll value during the summer months and this, according to these authors, may be due to the presence of large numbers of flagellates which are not preserved after fixation. Conover (1956) based on the observations of Atkins and Parke (1951) further states that "a population containing large cells would show small cell numbers relative to chlorophyll while population containing small cells would give a large ratio of numbers to chlorophyll". Harvey (1953) also observes that differences in the physiological state of the cells can affect the amount of chlorophyll present. The same author (Harvey *et al.*, 1935) states that no exact relationship can be expected between the pigment and diatoms of greatly different sizes. However, a relationship could be worked out between cell numbers and chlorophyll if the cell size is also taken into account.

(d) Total biomass and total organic matter

Zenkewitch (1931) explained the biomass as the quantity of living substance of organisms per unit volume. The measurement of the quantity of living substance by weight—biomass—is very useful in comparing productivity of different seas. The total biomass of a community is the community biomass which is the sum total of many species biomasses. Allee *et al.* (1950) have pointed out that we have no complete biomass data for any given community and the best approach at present to community biomass is that of Juday (1942). No work on the total biomass and total organic matter has been done in the Indian Ocean except that of Thompson and Gilson (1937) for a few stations during the " John Murray Expedition". The total biomass and total organic matter values obtained during the period of study are given in Table II and shown in Fig. 3.

A maximum of 3107 mg./M^3 of total biomass was observed on 16–2-1956. The monthly maximum value of 1230 mg./M^3 and a minimum of 123 mg./M^3 of sea-water were observed during February and January respectively. The monthly average values of organic matter ranged from 712 mg./M^3 during February to 28 mg./M^3 of sea-water during January 1956.

From the figure and table showing the total biomass and total organic matter variations, it may be seen that there is general agreement between these values and total plankton production. A major peak of biomass was observed in February and a minor peak in November. The lowest values were obtained in January and August, preceding the major and minor peaks respectively. The total organic matter closely followed the total biomass values with their minimal values in January and August.

Discussion

It is well known that the productivity in any particular zone of the sea is assessed by the estimation of the standing crop which in its turn is largely influenced by the prevailing hydrographical conditions, during the various seasons. From the data presented already it is clear that the production of phytoplankton at the fixed station in Lawson's Bay shows a major peak in the March to April period and a minor bloom in October and November. The zooplankton shows a major peak more or less coinciding with the major phytoplankton peak.

It has been already stated in an earlier part of the paper that an inverse relationship between temperature and dissolved oxygen and a direct relation between the phytoplankton production and oxygen were observed during the January to June period. Jayaraman (1951) indicated dissolved

oxygen as "a fairly good index of the activity of the phytoplankton" while Ramamurthy (1953 *a*) working in the same area, off Madras, states that "the oxygen content was mainly influenced by water temperature rather than by photosynthetic activity". During the July to December period there is a steady increase in oxygen except in November, which may be due to the high dilution consequent on the influx of considerable volumes

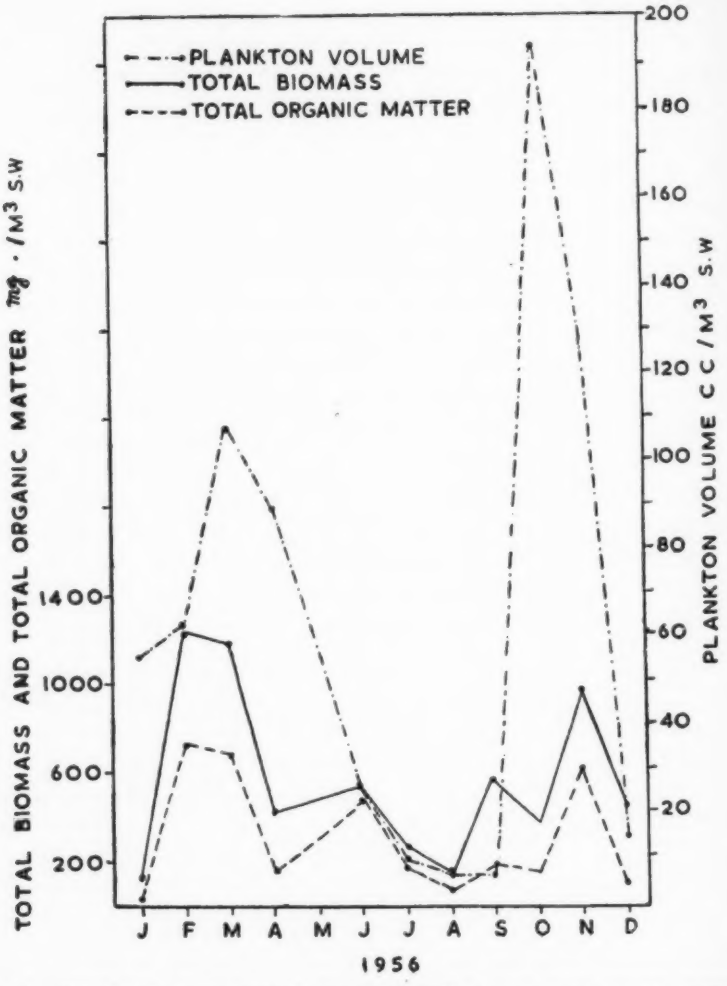

FIG. 3. Showing the mean monthly values for total biomass, total organic matter and lankton volume in the surface waters off Lawson's Bay during the year 1956.

of river water. The presence of a large population of photosynthetic organisms which pass through the ordinary filtering net used has been suggested by Graham (1943) to account for the excess of oxygen for some of the northern stations in the Pacific where the phytoplankton population was very scanty. Raghuprasad (1956) did not find any consistent relationship between quantities of phytoplankton and oxygen saturation off Mandapam, in the Gulf of Mannar, while Kasturirangan (1957) found off the Malabar coast a correlation between high phytoplankton production and high oxygen values during the monsoon months June to July and August.

It is generally believed that the nutrients will be low during periods of intense phytoplankton production. This assumption is largely based on work carried out in temperate waters. Our observations showed that there is no inverse relationship between nutrients and the phytoplankton. Raghuprasad (1956) at Mandapam and George (1953) at Calicut did not notice any such inverse relationship. Ramamurthy (1953 *a*) observed in Madras waters no well-marked seasonal cycle in the phosphates coincident with the absence of a marked seasonal growth in diatoms. Graham (1943) has drawn attention to the fact that any correlation between phytoplankton production and abundance of nutrients would largely depend on the stage of development of the phytoplankton pulse. He has described three such distinct stages, an initial one when there is sparse phytoplankton population and high concentration of nutrients, a middle stage where there is dense phytoplankton population and high concentration of nutrients and a final stage where there is dense phytoplankton population but low nutrient concentration. According to him there is direct relationship between density of phytoplankton and concentration of nutrients only in the middle stage of the pulse whereas in the initial and final stages the relationship is inverse. A fourth stage when there is a high concentration of nutrients in the surface layers, owing to active regeneration from the organic detritus, followed by a minor phytoplankton pulse has also been indicated by Graham. These phytoplankton pulses according to the same author (Graham, 1943) " can run its course only when there is a single supply of nutrients which is not augmented during the pulse " which is characteristic of cold temperate sea regions. Judging from our observations, during the major phytoplankton bloom off this coast, we have found that the correlation between the phosphate values and the phytoplankton density followed more or less on the lines indicated by Graham (Fig. 4). The first stage of high phosphate content and low phytoplankton population was observed in the third week of March followed by a rapid rise both in phosphates and phytoplankton towards the end of the month. These two stages correspond to the first

two stages described by Graham. The third stage when the phosphate values came down while the phytoplankton values continued to be high was observed in the first and second weeks of April. However, the phosphate value was appreciably higher than one would expect during the period, which is due to the upwelling of the bottom waters already reported by us (Ganapati and Subba Rao, 1957). A small increase in phosphates was observed in the third week of April presumably owing to regeneration, while the phytoplankton density continued to drop. A slight pulse in the

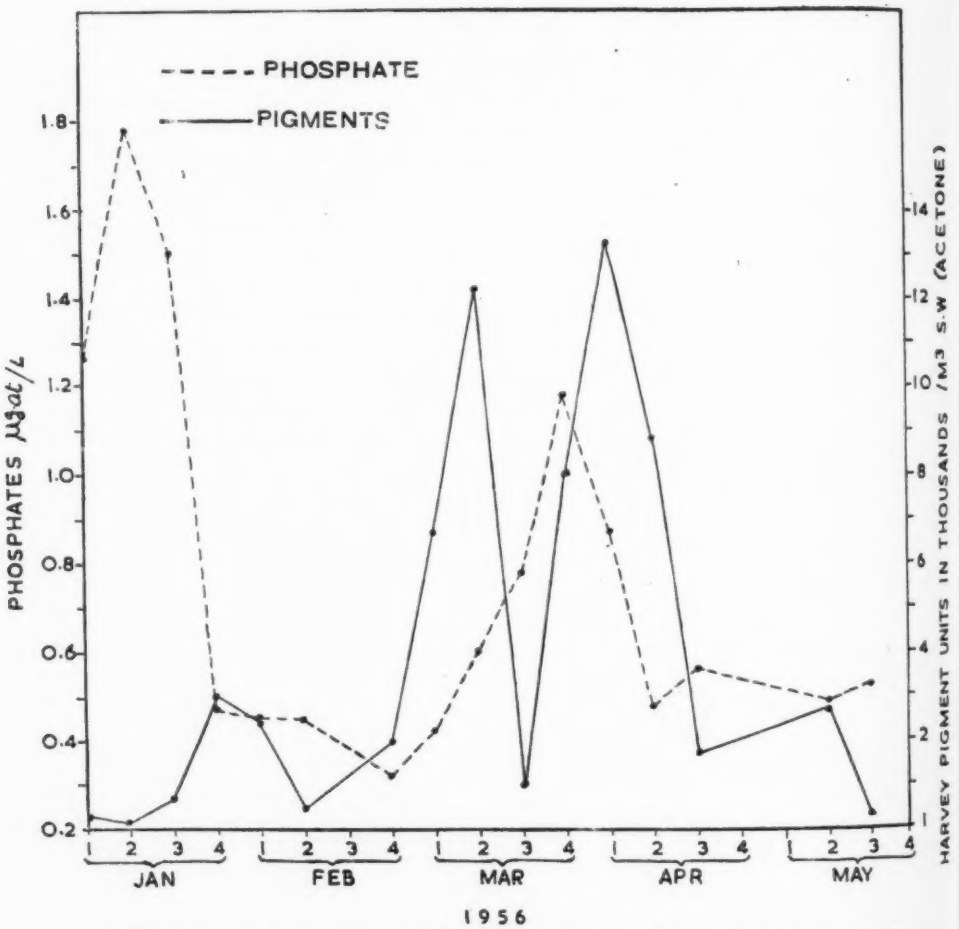

FIG. 4. Showing the mean weekly values for phosphates and pigments in the surface waters off Lawson's Bay during the period January to May 1956.

phytoplankton was observed in the second week of May soon followed by a rapid drop. This stage may be compared to the fourth stage described by Graham.

The zooplankton numbers were at their maximum when the temperature was low and the salinity high. They were in poor numbers during October and November when the salinity was at its minimum and the temperature fairly high. The Chætognaths off this coast were found by Ganapati and Satyanarayana Rao (1954) to occur in their maximum numbers during the month of March when the salinity was high and the temperature low. The same authors (Ganapati and Rao, V. R., 1954) found that the copepods also showed a similar relationship to the hydrographical conditions. Ramamurthy (1953 *a*) off Madras, and George (1953) off Calicut, observed similar relationships between the copepods, salinity and temperature. Raghuprasad (1956) found no consistent relationship between salinity and zooplankton. While at station G in the Gulf of Mannar, this relationship was inverse, a direct relationship between the two was observed at station P in the Palk Bay.

Ganapati and Rao (1953) observed that there is a regular sequence in a north to south direction in the commencement of the primary maximum of phytoplankton on the east coast of India. At Waltair it commences in February, at Madras during March (Menon, 1931) and at Krusadai in the Gulf of Mannar during June (Chacko, 1950). From the observed data on the surface salinity conditions at Waltair and Madras it is interesting to note that there is a time lag in a south to north direction of the maximum salinity values at the two stations during the northerly current period (January to June). At Madras it is during March to April (Jayaraman, 1951; Ramamurthy, 1953 *a*) and at Waltair the maximum values are during the April to May period. The time lag noticed for the maximum salinity values depends to some extent with the distance and the speed of the current. The succession of the phytoplankton blooms observed by Ganapati and Rao (1953) is from north to south whereas the current is from south to north during the same period as indicated by the salinity succession. Riley and Conover (1956) at Long Island Sound studied the distribution of the phytoplankton in relation to the hydrographical conditions. Based on the chlorophyll values they found an east-west gradient the maximum concentration being observed in the north-west part of the Sound. With respect to nutrients and salinity they have obtained the opposite situation. As we have at present no data on the nutrient gradients for the other centres on the east coast for the same period, it is not possible to generalise

the reasons for this succession in a direction opposite to that of the prevailing current.

It may not be out of place to mention about the succession of the salinity and the silicate values at the same centres in a north to south direction during the southerly current period, July to December. Owing to the influx of large volumes of river waters from the north the salinity values are very low and the silicate values very high. The influx of the fresh-waters during the southerly current was first felt at Waltair during October, then at Madras during November (Jayaraman, 1951 and Ramamurthy, 1953 *a*) and lastly at Mandapam during January (Jayaraman, 1954). Nash (1947) suggested that the silicates can be used as a tracer of fresh-water just as salinity is used as a tracer of the sea-water carried up the estuary. From the above observations it can be suggested that the high silicate values together with the low salinities can be used as tracers of fresh-water. The influence of the fresh-waters from rivers to the north during the southerly current is greater than the incursion of the oceanic waters during the northerly current.

Lebour (1917) and Herdman (1922) and several other workers have observed in the temperate seas a regular succession of the maxima of the three major components of the plankton, namely, spring and early summer maximum for diatoms, a summer maximum for dinoflagellates and early winter maximum for copepods. From the data presented by us it may be observed that there is a spring diatom outburst with which both the dinoflagellate maxima coincide (Table II, Fig. 5). Off Madras coast also the diatom and dinoflagellate maxima are reported to coincide (Menon, 1931; and Ramamurthy, 1953 *a*). Marshall, Nicholls and Orr (1934) investigating the biological conditions of the Clyde Sea area found that the spawning of *Calanus finmarchicus* coincided roughly with the diatom flowerings. The copepod succession after diatoms need not be the case always as observed by Jacob Somme (1934) in Norway, Clarke and Zinn (1937) at Woods Hole and Wimpenny (1936) in the North Sea. Wimpenny (1936) points out that " the metabolism of the thickly massed plant cells might be expected to produce an environment attractive to animals ready to spawn and favourable for the development of the newly hatched young ". Some investigators like Fish and Johnson (1937) and Bigelow (1926) have reported that the copepod populations are present in areas where diatom flowerings were seen. Menon (1931) and Ramamurthy (1953) off Madras, and Raghuprasad (1954, 1956) in the Gulf of Mannar, for Station G, have observed an inverse relationship between phytoplankton and zooplankton. On the

contrary Raghuprasad (1956) observed during most of the period at Station P, a direct relationship between phytoplankton and zooplankton. Fleming (1939) is of the view that there is little likelihood of finding a large zooplankton population in association with dense diatom population if the samples are

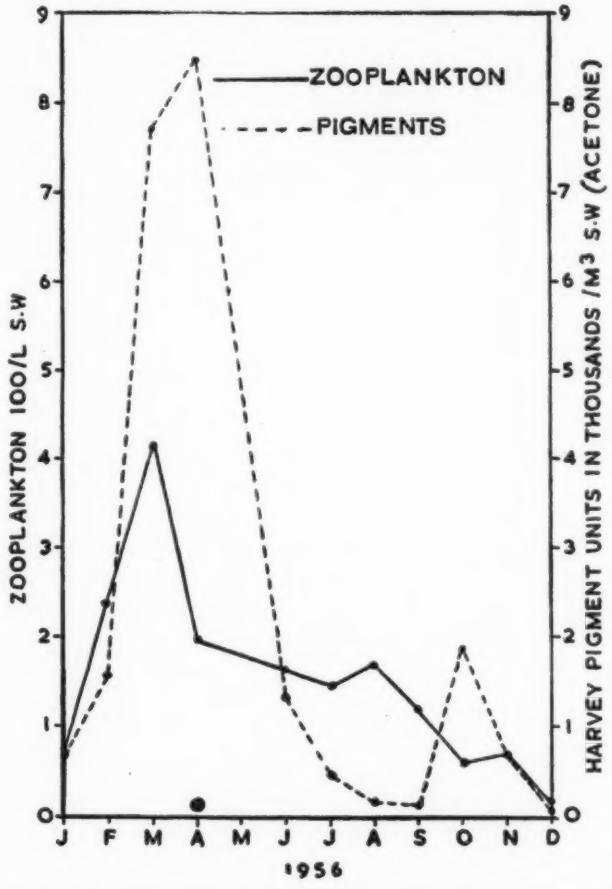

FIG. 5. Showing the mean monthly values for pigments and zooplankton organisms in the surface waters off Lawson's Bay during the year 1956.

collected at closer intervals. The abundance of the invertabrate larvæ, however, coincided with the phytoplankton peaks indicating a nutritional relationship between the larvæ and the phytoplankton. A similar relationship between invertebrate larvæ and phytoplankton have been reported

by Aiyar (1936) and Ramamurthy (1953 *a*) off Madras and Raghuprasad (1954 *a*, 1956) in the Gulf of Mannar and George (1953) off Calicut.

From the above discussion it may be concluded that the productivity of the local waters is mainly controlled by the two currents. The hydrographical conditions are more favourable and stable during the northerly current system in the January to June period when there is enrichment of the surface waters by upwelling of the subsurface waters. The northerly current also brings in the enriched oceanic waters of the bottom Antarctic drift which upwells near the Carlsberg ridge and bifurcates into two branches one of which enters the Bay (Sewell, 1932). During the southerly current system, from July to December, the inshore waters in the Bay present nearly estuarine conditions and the general low production during the period may be attributed to the drastic fluctuations in hydrographical conditions. There is, of course, enrichment of the nutrients which are washed down by the great rivers to the north and a minor peak of phytoplankton is observed in the October to November period.

SUMMARY

Observations on the hydrographical and planktological conditions of the surface waters at a fixed station located at the 10 fathom line in the Lawson's Bay, Waltair, have been made for the year 1956.

The influence of the prevailing currents on the hydrographical conditions in the inshore waters of the Bay of Bengal is discussed. A north to south succession of the periods of low salinity and high silicate content at three stations Waltair, Madras and Mandapam on the east coast of India has been observed during the southerly current period, July to December. A similar succession of the high salinity values in a south to north direction during the northerly current period, January to June, has also been observed at Madras and Waltair.

Quantitative plankton estimations have been made by counting, volume estimation, pigment extraction, total biomass and total organic matter for known volumes of water. There was general agreement between the data using the different methods.

A major phytoplankton peak during March to April and a minor phytoplankton peak during October to November were observed. The diatom and dinoflagellate maxima coincided with each other.

Pigment extraction was made with three different solvents and it was found that ethyl alcohol-benzene mixture was a better solvent than acetone and methyl alcohol.

A general discussion is given on our observed data of plankton production in relation to the hydrographical conditions and inferences drawn in the light of similar investigations from Indian waters and elsewhere.

Acknowledgments

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INTER-PETIOLAR STIPULES OF *GALIUM MOLLUGO* L.*

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Abstract

The inter-petiolar stipules of *Galium mollugo* are separate, leaf-like in shape and size, and also in the possession of a single trace bundle. Morphogenetical studies at the shoot apex show that the initiation and vascularization of the leaves and stipules are different. Only the leaves bear axillary buds; the leaf-like stipules do not bear them. The foliar foundations by their union form a *collar* round the axis, from which it separates by the differentiation of a separation layer in its adaxial surface. The glandular hairs also arise from this layer. The axis on its separation shows a wavy contour of its surface. The crest of each initially shallow wave is occupied by a meristem which helps in the development of each ridge. From later behaviour of the apparently single leaf-trace bundle it appears that it is composed of three bundles and the laterals are concerned with the origin of the stipules and their traces.

The family Rubiaceæ, to which *Galium mollugo* belongs, have stipules as a diagnostic character. The inter-petiolar stipules of the family show a variety of forms. 'The two stipules, one from each leaf, that stand side by side, are usually *united*,' as in *Pæderia fœtida* and *Ixora parviflora*; or they may remain *separate*, as in *Galium mollugo*. There occurs another form, the *intra-petiolar*, in which the stipules unite between the petioles and the axis, ' and are frequently united to one another and to the petiole so that a *sheath* is formed round the stem,' as in *Gardenia*.

In *Galium* the stipules remain separate, in size and shape they resemble the leaves for which they are often mistaken. This happened when Hooker (1875, p. 22) stated that in the Rubiaceæ the leaves are stipulate except in the Galieæ which are without stipules.

In *Galium mollugo* the leaves and stipules are slightly stalked, 6–8 in a whorl (Fig. 1). When the stipules are separate and leaf-like, the number of such leaves and stipules should be 6 (2 + 4), but in *Galium* the number

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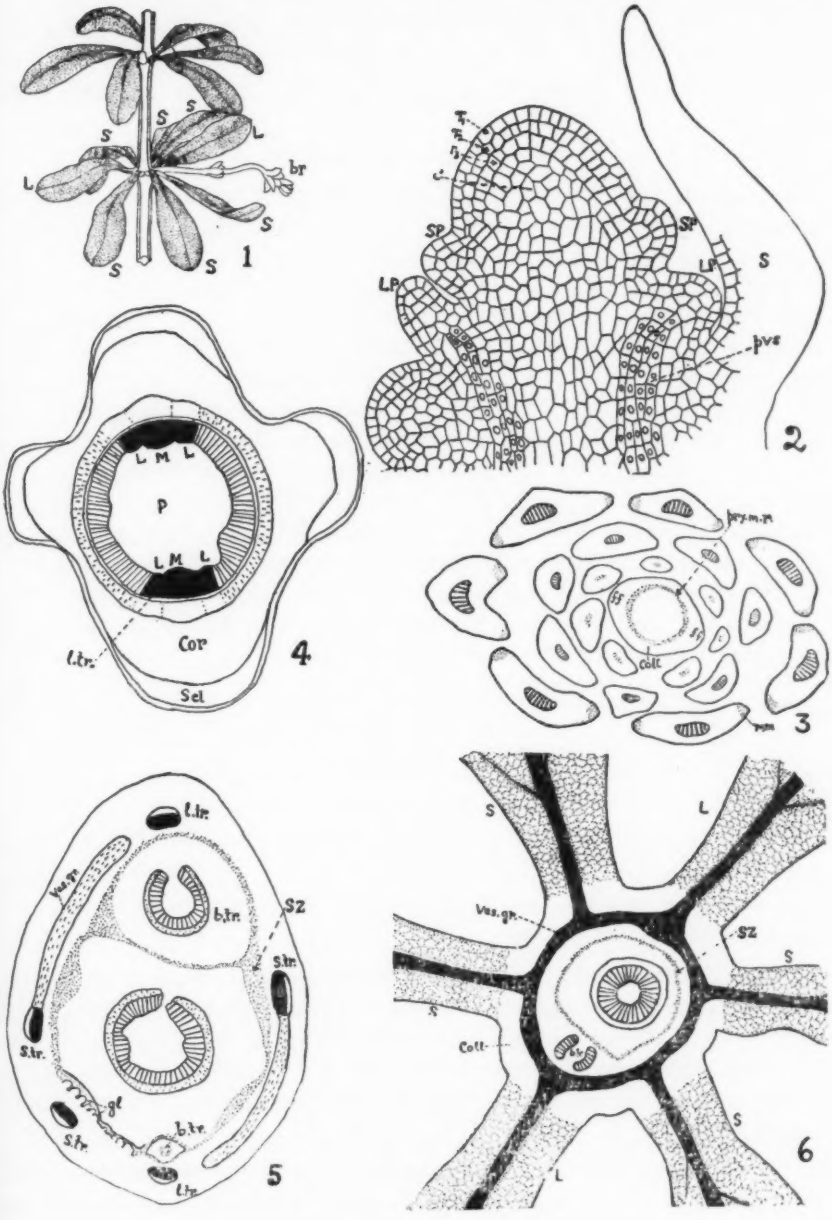
varies from 4 upwards (Strasburger, 1908; Lawrence, 1951). Willis (1951, pp. 573–74) offered an explanation for the reduced and the higher number of stipules in this species. He writes: The real leaves are those which subtend axillary buds. In the simplest case of a whorl of 6 each leaf has 2 separate stipules; if the stipules be united in pairs a whorl of 4 results; if each of the stipules be branched into 2, we get a whorl of 10; and if the centre pair of half stipules on either side be united a whorl of 8 results. In *Galium mollugo* a whorl of 7 makes the above explanation rather difficult to accept without their ontogenetical studies.

In 1914 Sinnott and Bailey while investigating on the phylogeny of the Angiosperms studied the nodal anatomy of a very large number of species under different families and suggested a clue for the determination of the morphology of the stipules. They stated that " in the majority of plants with a *trilacunar node*, stipules are present; that in almost in all with *unilacunar node*, stipules are absent, ..." (p. 442). They stated further that " each stipule obtains its vascular supply, if it has one, from branches derived from the corresponding lateral traces " (p. 447).

In the Rubiaceæ the node is generally unilacunar, and as such, the leaves should be exstipulate. Sinnott and Bailey explained this nonconformity by stating that " in the Rubiaceæ although the vascular supply causes a single gap the leaf-trace is composed of three bundles; and the stipules when present are often related to the lateral ones of this trio " (p. 449). In regard to the nature of the inter-petiolar stipules of this family they stated that these are " the products of a fusion of two formerly independent and adjacent ones; since each stipule receives its vascular supply from both leaf-traces " (p. 449).

Mitra (1948) worked on the origin, development and morphology of the inter-petiolar stipules (united) of *Pæderia fœtida* (Rubiaceæ). The leaf-trace in this species consists of two bundles placed very close to each other while being differentiated in the primary meristem ring (Helm, 1931). But before leaving for their leaf they unite, and on leaving cause *only one gap in the nodal ring*. The two fused bundles however, even before they leave the stele, show organization into three regions: a central and two laterals. Only the central one goes to the leaf as its median bundle, but the two laterals diverge around the axis through the tangentially extended foliar foundations (*collar*); the corresponding laterals from the opposite leaves meet and fuse to form a closed circuit of provascular meristem in the collar. These combined laterals then give out branches which form the traces of the stipules which are composite in nature.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FIGS. 1–6.

The free stipules of *Galium* present altogether a different problem, and the present investigation has been made with a view to study the origin and vascularization of the stipules and leaves which appear morphologically alike.

MATERIALS AND METHODS

Materials for the study were collected in Darjeeling, and fixed and preserved in Formol-acetic acid-ethyl alcohol solution. Attention of the senior author was drawn to this species, *Galium molluga* L., by his host Prof. J. Saha of Darjeeling Government College, in April 1956. Sections, both longitudinal and transverse, were cut 8–10 μ thick, and stained in safranine-fast green combination. Freehand sections through the nodal regions of adult shoot were also cut, suitably stained, and made permanent for a comparative study of the nodal structures in the young and adult shoots. To study the vascular supplies to the leaves and stipules from the axial ring *in situ* suitably thick nodal region with intact leaves and stipules was cut from the stem and treated with lactic acid at ordinary room temperature for four days to make the region perfectly clear; equally good result was obtained by heating the sections in lactic acid at 60 ° C. for 2-4 hours. The cleared material was then thoroughly washed and lightly stained with safranine solution, washed again and mounted in 50% glycerine solution, and finally sealed with paraffin wax.

The text has been illustrated with camera lucida drawings and photomicrographs; duplication has been avoided. Magnifications have been noted for each drawing in the explanations.

OBSERVATIONS

1. *Shoot Apex Organization.*—The free apex is dome-shaped measuring about 60 μ high and 75 μ wide at the level of divergence of the youngest pair of leaf primordia (*see* Table I where measurements of six apices in longitudinal sections are given).

The tunica consists of 2–3 layers of anticlinically dividing cells running over the dome, and encloses the corpus consisting of a mass of irregularly dividing cells, larger in size with contents which stain lightly. Regular zonation of the apical meristem into distinctly discernible regions, like the central mother initials, flank meristem and file meristem, is not evident (Fig. 2, 7).

2. *Initiation of the Leaf Primordia.*—About 32 μ below the extreme tip the shoot apex in transverse section shows a rounded mass of cells with two distinct regions: a central lightly stained and a peripheral deeply stained

TABLE I

Number of shoot apices	Height in μ	Width in μ
1	45*	75
2	60	75
3	60	105†
4	64	75
5	65	80
6	62	74

* This may be from a tangential l.s.

† This evidently includes the foliar foundations of the next pair of leaf primordia.

more compact cells corresponding to the corpus and tunica areas (Fig. 9). 24–30 μ further down the apex, *i.e.*, 56–62 μ below the extreme tip, the initiation of a pair of leaf primordia is indicated by the horizontal extensions of the axis giving the latter an oblong form (Fig. 10). These local extensions of the axis upon which the foliar erection is later seen to take place has been named by Grégoire (1935) and Louis (1935) *soubassements foliares*, translated *foliar buttress* by Foster, and *foliar foundation* by Majumdar. The formation of this foliar foundation at the shoot apex prior to the erection of the leaf primordium upon it has been confirmed by later workers on morphogenesis at the shoot apex of dicotyledons (Foster, Majumdar, Mitra and others).

The foliar foundations, formed in definite sectors of the apical dome and placed almost opposite to each other, then extend tangentially around the dome by divisions of the hypodermal cells until the two corresponding extending arms meet and fuse to form a *collar* congenetically united with the axis (Figs. 3, 5, 10). Meanwhile a primary meristem ring is seen to organize in the apical meristem in which the acropetally progressing trace bundles soon differentiate (Figs. 3, 11).

Further down the axis the apical bud in transverse sections shows the leaves and stipules arranged in different stages of development and separation from the axis and from one another. The primordia of the leaves and stipules also show stages in the development of the lamina from marginal

meristems with very feeble secondary veins (Figs. 3, 6, 15–17). It will be seen from Figs. 17 and 18 that the stipules and leaves with their single midrib bundle cannot be distinguished one from the other except for the fact that the leaves bear vegetative buds in their axils and the stipules are without them. Only one of the two axillary buds at a node grows into a full-grown branch (Figs. 1, 15–17).

In median longitudinal sections of the apical bud the leaves and the stipules could also be distinguished. The leaves get their vascular supply directly from the axial ring, but the stipules get their supply secondarily from the girdle formed by the fusion of the laterals (?) of the leaf traces (*see* below. Figs. 6, 8, 14).

3. *The Leaf-trace.*—In *Galium* apparently only one provascular strand for each leaf primordium differentiates in the nodal ring. At a later stage of differentiation each such strand is seen to organize into three recognisable regions, namely, a central flanked by two lateral ones (Figs. 4, 12, 13). The central one later moves out towards the base of the petiole and form the single median bundle of the leaf. The laterals instead of following suit turn round and diverge in the collar along the sides of the axis, meet the corresponding ones coming from the other leaf, and coalesce to form a *complete girdle* or circuit of vascular meristem running from one primordium to the other. The two median strands stand out facing each other as two prominent knots in the girdle (Figs. 6, 14).

4. *The Stipular Trace and the Origin of the Stipules.*—In the portion of the girdle between the two median strands the trace of each stipule differentiates at equal intervals. If the two leaves are placed at the node just opposite to each other the number of stipule traces is equal on each side (*i.e.*, 2, 2 or 3, 3); but if their positions are not quite opposite then the number of such traces are unequal, *i.e.*, 2 and 3, on the two sides (Figs. 5, 18). This explains the number of leaves and stipules, 6, 7 and 8, found in a whorl in *Galium mollugo*. Increase in the number of stipules by branching or splitting has not been observed in this species. The traces show equal development in both the structures. Each stipule primordium is organized by the active division of the cells surrounding its trace.

5. *Separation of the Collar from the Axis.*—A zone of actively dividing cells appears at the junction of the collar and the axis still confluent with each other. The origin of this zone takes place in the cells of the adaxial surface of the collar (Figs. 5, 13, 14).

Separation takes place in this zone. One very interesting fact is noticed: this layer also gives rise to the appendages, the glandular hairs. These are found borne on the adaxial surface of the collar and stipules and of the leaves, but in the case of the latter they are borne near the margins, the region opposite the axillary bud which separates from the axillant leaf very late, is devoid of these glandular hairs. Separation of the collar and the development of the glandular hairs appear to have some intimate connection as they occur almost simultaneously (Figs. 5, 15).

The separation layer does not pass round the axis in a circular way. It is rather wavy in outline passing internal to the axillary buds and below the collar, so that when the axis separates it shows four or five irregular shallow ridges on its surface. A part of the separation layer also passes external to the axillary bud and later causes separation of the latter from the former (Figs. 5, 6, 13).

6. *Separation of the Leaves and Stipules from one another.*—The beginning of their separation from one another is almost simultaneous with the separation of the collar from the axis. Upgrowth of the leaf and stipule primordia is maintained by active divisions of cells immediately surrounding each trace bundle, and then by intercalary growth of each primordium. The beginning of isolation of each primordium, as a result of this local growth, is indicated by the appearance of notches or furrows on the outer surface of the collar demarcating the area of individual pieces. The furrows then proceed inwards ultimately dividing the collar into as many parts as there are leaves and stipules in a whorl (Figs. 15, 16, 17).

At separation the base of the petiole of each primordium is convex on the abaxial surface and almost flat on the adaxial, and angular where these two surfaces meet. The petiole continues as the midrib region of the blade. Its angular portions are occupied by marginal meristems which are responsible for the development of the wings of the lamina. One or two feeble branches are given out by the midrib bundle into the developing wings (Fig. 6).

7. *Appendages of the Stipules and Leaves.*—Their origin has already been described. They fill up the space formed between the stipules and the axis during its separation from the collar. These hairs have also been described and figured by Metcalfe and Chalk (1950). According to them they are composed of resiniferous cells. Their structures have well been described by Mitra (1948) in the cases of *Pæderia* and *Ixora.*

8. *Ridges of the Internode.*—The adult internode has four or five well developed ridges and as many furrows (Fig. 19). They are without any

vascular tissue, and the summit of each is occupied by a group of cells which are thick-walled and sclerenchymatous. The epidermis is provided with a few short and stiff unicellular hairs. Both these features give the stem its rough exterior.

At the node the ridges and furrows are absent. The manner of separation of the collar from the axis gives the latter a wavy contour. The crest of each wave is occupied by a meristem which by centrifugal differentiation causes its radial extension and a high ridge is formed. The cells at the summit of each ridge are finally modified into a mechanical tissue.

DISCUSSION AND CONCLUSION

The origin and morphology of the stipules have been a subject of enquiry since perhaps the days of Henry (1846). But the three outstanding contributions to this subject came from Eichler (1861), Colomb (1887) and Sinnott and Bailey (1914). Eichler was the first to point out that the stipules arise without exception in pairs secondarily from the leaf-base by intercalary growth. Colomb then established the intimate association of the origin of the stipules and their traces with the branches of the laterals of a three-bundle leaf-trace. Sinnott and Bailey from very wide and exhaustive studies of the Angiosperms came to the conclusion that:

(1) Plants with unilacunar nodes are mostly without stipules,

(2) Plants with trilacunar nodes are mostly stipulate, and

(3) the origin of the stipules and their traces is invariably correlated with the branches of the laterals of a three-bundle leaf-trace.

Mitra in a series of papers on different types of stipules, *viz.*, free lateral, inter-petiolar, intra-petiolar, adnate, foliaceous, ochreate, etc., supported Colomb and Sinnott and Bailey in all essential details and Majumdar (1955) showed that the stipules in all cases are outgrowths of the base of the stipulate leaf and thus supported Eichler. But the best proof of "correlation between stipules and lateral leaf-traces" came from Dormer (1944) and Sensarma (1957).

In *Lotononis corymbosa* (Leguminosæ) and *Azara microphylla* (Flacourtiaceæ) in which the stipules are single and of the same size and shape as the leaves, the leaf-trace consists of two bundles, the median and a lateral, the second lateral evidently is suppressed. Dormer explains that "the bilacunar condition is the result of suppression of one stipule and of the corresponding lateral trace". The conclusion is rather indirect, but Sensarma has provided direct evidence from a study of the origin and vascularization of the stipules in *Muntingia calabura* L. (Elæocarpaceæ). The node

in this plant is trilacunar, and the leaf-trace consists of three bundles—the median and two laterals. One of the laterals branches and a stipule is formed on that side with the branch as its trace, whereas the other lateral does not branch and a stipule is not formed on that side. Therefore, the absence of a stipule is definitely correlated with the absence of branching of the second lateral.

In both *Pæderia* and *Galium* from its very differentiation the leaf-trace consists apparently of one bundle, and the node is unilacunar. The subsequent behaviour of the leaf-trace, however, shows that it is composed of three parts (*cf.* Sinnott and Bailey). Sinnott (1914) postulated that the trilacunar condition of the node is the primitive condition, and the unilacunar one has been derived from it either by the suppression of the two laterals, or by their approximation with the median. Studies on the nodal structures of the above species appear to support the origin of the unilacunar condition by the approximation of the three trace bundles into one, and the stipule traces are derived from the branches of the laterals.

Separation of the collar (fused foliar foundations at the node) from the axis is effected by the origin and differentiation of a *separation zone* in the adaxial surface of the collar. This meristem also gives rise to the glandular hairs.

The primordia of the leaves and stipules organize in the tissues of the collar immediately surrounding their individual trace bundle. Their upgrowth is maintained by active divisions of cells constituting each primordium. The lamina in each case is formed by a marginal meristem. Separation of each primordium from its neighbours begins in the form of notches or furrows on the outer surface of the collar between contiguous primordia. The furrows proceed inwards until the primordia are separated from one another.

Suggestions of Willis that the extra number of stipules above the normal four originate by their branching, or by branching and union, is not supported by the present developmental and anatomical studies.

Presence of glandular hairs on the stipules appears to be a regular feature in the Rubiaceæ. Their development has intimate connection with the separation of the collar from the axis. Their function seems also to be ecological.

The formation and growth of the ridges on the internode is determined by two factors, *viz.*, (1) the nature of the origin of the separation layer in the collar and internal to the axillary buds, and (2) the differentiation of

a meristem at the head of each ridge. At its separation from the collar and axillary buds, the axis shows a wavy contour of its outer surface. The meristem by cutting cells centrifugally adds to the height of each ridge. Finally the meristem is modified into a mechanical tissue surmounting each ridge.

Sinnott and Bailey have suggested that the stipules are homologous with a pair of earliest teeth, or the basal lobes of a leaf. From the nature of the vascular supply to these organs (teeth, lobes and stipules) from the trace bundles, the suggestion of these two authors merit further developmental studies which the present writers propose to undertake.

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Explanation of Text-Figures

Figs. 1–6.—Fig. 1. A portion of the adult shoot showing leaves (*l*) and stipules (*s*) in whorls at nodes; only one branch (*br*) is seen developed, ×1. Fig. 2. Median longisection of the shoot apex showing distribution of tunica (*t*) and corpus (*c*) in the apical meristem; leaf (*lp*) and stipule (*sp*) primordia; provascular strand (*pvs*) going to leaf primordia from the axial ring ×270. Fig. 3. Transverse section of the vegetative bud showing leaf and stipule primordia at different stages of growth and differentiation; in the central region the axis is seen completely surrounded by the foliar foundations (*ff* and *coll*) with a primary meristem ring (*pry.m.r.*) differentiated, ×95. Fig. 4. Transverse section of an internode just below the node showing the leaf-trace (*l, m, l*) just before leaving the axial stele for its leaf, ×20. Fig. 5. Transverse section of a very young node showing that the trace bundles have already moved out in the collar; the girdle (*vas.gr.*) is broken into discrete strands—leaf (*l.tr.*) and stipule (*s.tr.*) traces with a portion of the girdle still remaining; separation zone (*sz*) is differentiated and passes round the axis in a wavy manner; glandular hairs (*gl*) are seen to form the separation zone on the adaxiar surface of the collar; only one axillary bud develops, ×15. Fig. 6. Lactic acid treated nodal region in t.s. showing the connection of the leaf and stipule traces with the vascular girdle; separation zone, bud trace and axial stele are also shown, ×18.

br–axillary branch; *b.tr.*–bud trace; *c*–corpus; *coll*–collar; *cor*–cortex; *ff*–foliar foundations; *gl*–glandular hairs; *l*–leaf; *lp*–leaf primordium; *l.tr.*–leaf-trace (*m, l* are its median and lateral bundles); *mm*–marginal meristem; *p*–pith; *pry.m.r.*–primary meristem ring; *pvs*–provascular strand; *s*–stipule; *scl*–sclerenchyma strand; *sp*–stipule primordium; *s.tr.*–stipule trace; *sz*–separation zone; T_1, T_2 and T_3–tunica layers; *vas.gr.*–vascular girdle in the collar.

Explanation of Plate XXII

Fig. 7. The shoot apex in median longisection—it shows the origin of leaf and stipule primordia, vascular supply to the former, and origin of the glandular hairs (*cf.* Fig. 2).

Fig. 8. Vegetative bud in median longisection—shows the distribution of the leaf and stipule primordia and their vascular supplies; only one axillary bud has developed.

Fig. 9. T.S. of the shoot apex 32 μ below the extreme tip.

Fig. 10. T.S. of the shoot apex 65 μ below the tip—shows the formation of the collar by the foliar foundations still congenetically united with the axis.

Fig. 11. T.S. further down the shoot apex showing differentiation of the primary meristem ring.

Fig. 12. T.S. of the axis showing the leaf-trace organised into three regions, the laterals with a tendency to diverge from the normal course.

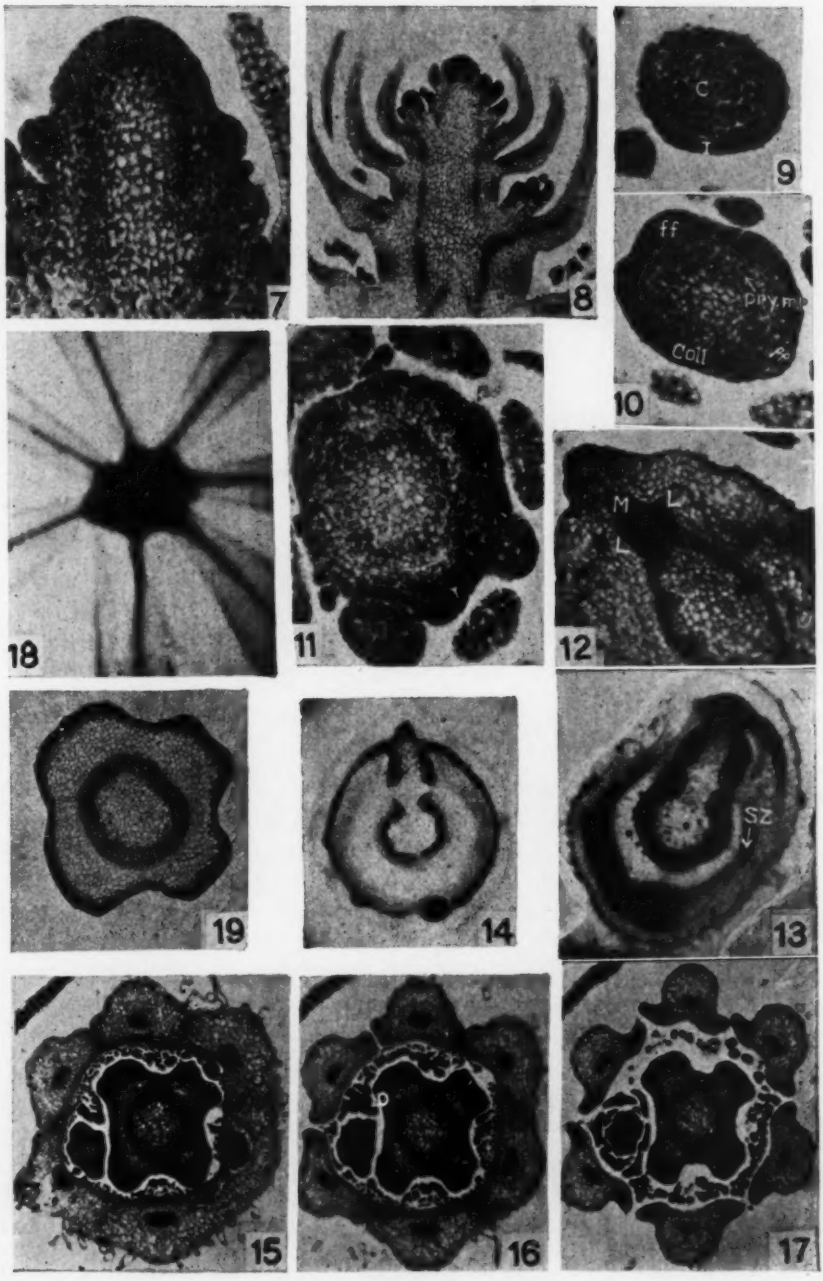
FIG. 13. T.S. of the axis with the collar showing the leaf-trace with the laterals diverged into the collar to form the vascular girdle, the bud trace and the separation zone passing round the axis.

FIG. 14. T.S. of the node showing axial stele, leaf gaps, bud-trace, leaf-traces forming a girdle around the axis with the traces of the pair of leaves and of the stipules in the girdle, and the separation zone.

FIGS. 15–17. T.S. through the node showing the separated collar with the leaf and stipule primordia organized around their individual traces; beginning and full separation of the primordia from one another; differentiation of marginal meristem in each primordium, position of the glandular hairs, formation of the lamina from the marginal meristem, development of only one axillary bud, the other atrophied (Figs. 16, 17); and the outline of the axis into broad furrows and ridge with meristems occupying apex of each ridge.

FIG. 18. Lactic acid treated nodal region showing the supply of the leaf and stipule traces from the vascular girdle of the collar (for details *see* Fig. 6).

FIG. 19. T.S. of adult internode.



FIGS. 7–19

STUDIES ON FOLIAR SCLEREIDS IN DICOTYLEDONS

VI. Origin and Development of Terminal Sclereids in the Leaf of *Niebuhria apetala* Dunn.

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This paper represents the continuation of the writer's studies on the ontogeny of terminal sclereids. Rao and Kelkar (1951) gave a detailed account of the polymorphic terminal sclereids in *Niebuhria apetala*. Recent studies have emphasised the need for an ontogenetic study to prove the real relationship of sclereids and the veinlet endings. The reported occurrence of terminal sclereids in *Niebuhria apetala* prompted me to make a detailed observation on their origin and development, an account of which is presented in this paper.

Leaf material of *Niebuhria apetala* was collected at the Indian Botanical Garden, Calcutta. The fixed material was dehydrated and embedded in paraffin according to customary methods. The technique of clearing the leaves is outlined in the previous paper (Rao, 1957).

Distribution of Sclereids in the Lamina

Cleared leaf preparations show that the sclereids develop in close contact with the vein-ends. Without any exceptions, the sclereids are terminal or sub-terminal in disposition. The sclereids are polymorphic and appear in groups of 2–5 often being superimposed. They are uniformly distributed in the sub-marginal region of the leaf, abundant near the midrib, and very rare near the margin. Besides sclereids, sometimes some of the vein-ends possess slightly sclerosed cells with abundant pits. Transections of the lamina show that the leaf is xerophytic and that the sclereids are conspicuous idioblasts closely attached to veins (Figs. 8, 10 and 11).

The sclereids are polymorphic and vary from a simple fusiform type to an asymmetrical type. They show a strong tendency to fork, leading to polymorphic shapes. The processes are very conspicuous being drawn out, sometimes into thin coiled structures (Fig. 10). Whatever their form, the sclereids have a thick wall and a large lumen. Under polarised light one

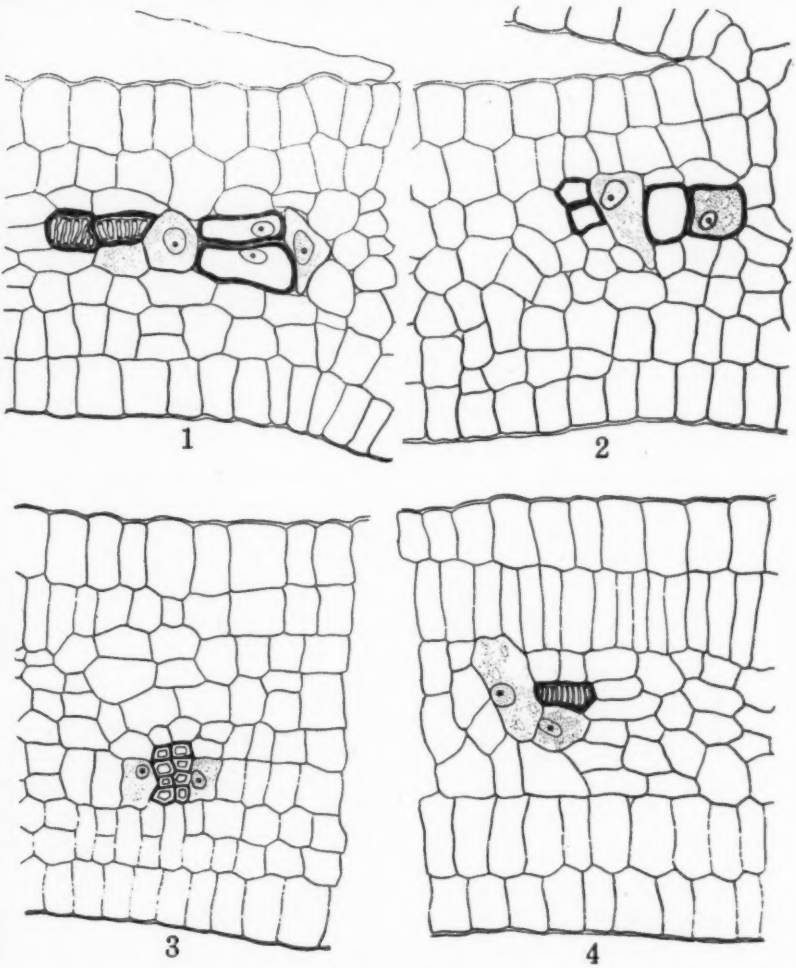
could see stratification in the thick wall and the presence of pit canals. The lumen is narrow or occluded.

ORIGIN AND DEVELOPMENT

The young unexpanded lamina is made up of compactly disposed parenchymatous cells with or without air-spaces. At this stage, one could see the differentiation of procambial cells in various degrees of development. Serial trans and paradermal sections at this stage reveal the presence of sclereid initials in close contact with the procambial cells (Figs. 1, 3 and 9). Such initials are present either solitary at the end of a terminus or in groups of 2–5 cells variously disposed and showing close contact with the vascular elements (Figs. 1, 2, 6 and 7). Irrespective of their place of origin, the initials show slight enlargement in the cell size with dense cytoplasm and prominent nucleus.

The sclereid initials are parenchymatous in form (Figs. 1 and 3), but as the development proceeds they acquire an irregular shape and size (Figs. 6 and 7). In the following stages, signs of growth are indicated by the psesence of slight processes (Figs. 2, 5, 6 and 7). Either they grow horizontally, acquiring a tubular form or vertically towards the adaxial and abaxial surfaces of the expanding lamina. The sclereid initiation and growth are not confined to the earlier phases of tissue expansion, but occur over extended period of time. With the development of sclereid initials the mesophyll exhibits differentiation into a well-defined palisade and a spongy region. To begin with, the palisade is one-layered, later on forming a compactly disposed double layers of cells as a result of a transverse division. The epidermal cells are well organised and exhibit a thick layer of cuticle with depressed stomata. The growing sclereid initials push their way amidst compactly situated cells. As observed in all growing sclereids, the growth is intercellular and bipolar and is confined to the tips only. Branching is a common feature, which is noticed in almost all growing sclereid cells. This results in the formation of variously forked bizarre cell forms. The sclereid processes are drawn out and in no instance was the extension near the upper epidermis noticed. In some sclereids the main body showed a regular T-shape. In advanced cases, the arms of T exhibit growth towards the spongy region in the form of well drawn out filiform processes (Figs. 8 and 10). Most of the sclereids show a great deal of extension and branching in the abaxial portion of the lamina. The horizontally disposed sclereid cells show a more or less rectangular form with vigorously drawn out processes. Sometimes, the end portion of the abaxial processes of the sclereids are situated in the respiratory cavity of the stomata. The growing cell has a smooth wall and is free from

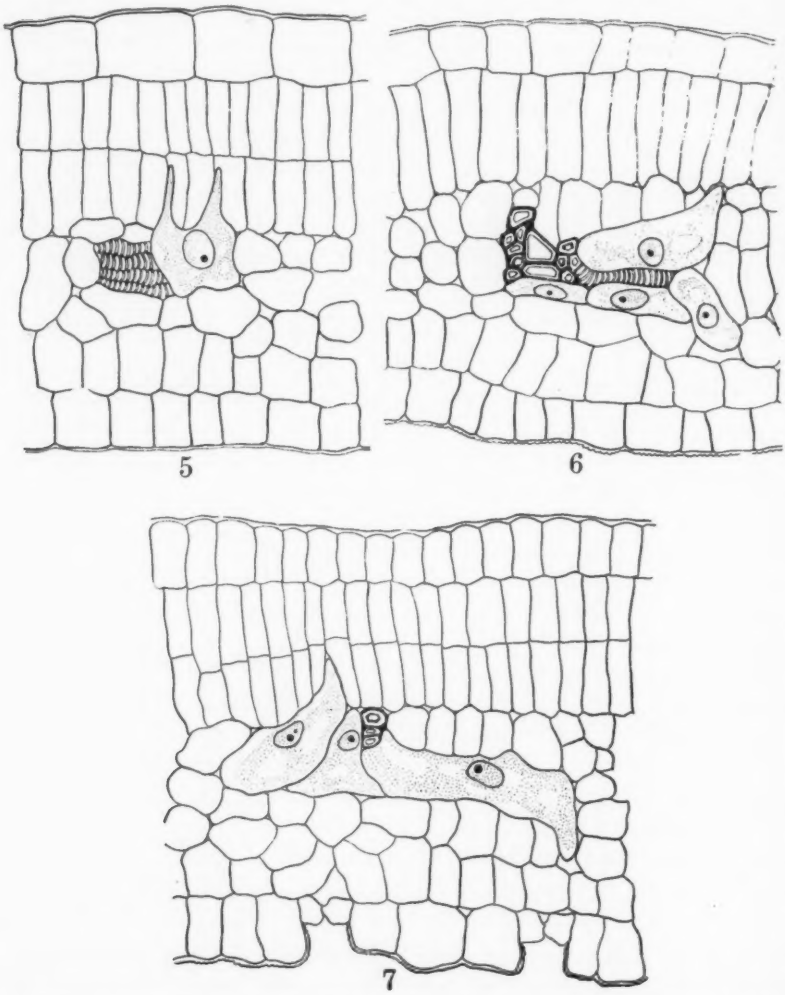
spicules. The nucleus varies from large spherical to pear-shape and occupies different positions in the growing cell. The growth of the cell seems to keep



FIGS. 1–4. Transections of young and immature leaves of *Niebuhria apetala* illustrating early stages in sclereid ontogeny, ×225 eacn. Fig. 1. Sclereid initials. Fig. 2. A young growing sclereid. Fig. 3. Sclereid initials on either ends of tracheary elements. Fig. 4. Sclereid initials and mature tracheidal cell.

pace with the rapid expansion of mesophyll cells. With the cessation of the growth of mesophyll cells, sclereid growth also stops and shows signs of

secondary wall thickening. The first visible signs are the slight thickening of the primary body; this is soon followed by depositions of lignin, more or less in a uniform way. In the final stages, they develop a massive wall,



FIGS. 5–7. Transections of young and immature leaves of *Niebuhria apetala* illustrating early stages in sclereid ontogeny, × 225 each. Fig. 5. Sclereid initial showing prominent processes. Fig. 6. Growing sclereid cells and tracheary elements. Fig. 7. The amœboid appearance of the growing sclereid. Note the entry of the distal part of sclereid into the air-space.

leaving a lumen of irregular width in the main body, while in the processes the lumen is very narrow or occluded.

The secondary wall shows striations and possesses numerous pit canals. The pits are often very closely disposed or may be distributed all over the surface. With the onset of sclerosis, the nucleus along with the protoplasts shows signs of degeneration. Ultimately the nucleus disintegrates with the formation of a thick wall in the mature sclereid. The process of sclerosis also seems to extend over a considerable period of time. First, those which are near the midrib and at the margins undergo sclerosis followed by those which are in the sub-marginal region of the lamina.

It is interesting to observe that the sclereid initial cell is terminal to the tracheid cell (Figs. 1 and 4), though this is not of frequent occurrence. At no time was the conversion of tracheidal cell into a sclereid seen, even though frequently they were found side by side.

Conclusion

Recent comparative studies in *Mouriria*, *Boronella* (Foster, 1946, 1955 *b*) and *Memecylon* (Rao, 1957) have shown the constancy of terminal sclereids as an important feature in any taxonomic study. A preliminary survey of cleared herbarium leaves of 4 species of *Niebuhria* has revealed that terminal sclereids are present only in *Niebuhria woodii* Oliver, whereas in *N. triphylla* Wendl., *N. rosmarinoides* Sind and *N. siamensis* Kurz. the leaves are free from sclereids and the vein-ends exhibit small irregularly-shaped storage tracheids.

In *N. apetala* and *N. woodii* besides terminal sclereids, sometimes some of the vein-ends possess storage tracheids with abundant pits. Intensive work on the other species of *Niebuhria* may reveal a wide range of cell forms. The present study also supports the view that a genus which has well developed terminal storage tracheids, will have some species or all species with well developed terminal sclereids.

It is of interest to record that in the lamina of *Niebuhria apetala* the origin and sequence of maturation of terminal sclereids and vein-ends exhibit a definite pattern. The sclereid initials originate progressively near the midrib and the margins and extend over to the remaining sub-marginal portions of the lamina. This exactly coincides with the order of maturation of the veinlet ends. The sequence of maturation of veinlet ends and sclereids thus follow the same pattern. This mode of development is in contrast with that of *Boronia serrulata* (Foster, 1955 *a*) where new sclereids originate progressively in basipetal sequence, coinciding with a similar order of maturation of the

veinlet ends. This emphasises the need for broad comparative ontogenetic studies.

Terminal sclereids during their growth exhibit long processes. It is not uncommon to observe the free ends of such processes in the respiratory cavity of the stomata. In some extreme cases as noted in *Boronia serrulata* and *Mourira huberi* (Foster, 1955 *a*; 1947) the sclereid tips push between guard cells.

SUMMARY

An ontogenetic study has revealed that sclereid initials in *Niebuhria apetala* are terminal or subterminal to the procambial strand. The origin and sequence of formation of terminal sclereids have been found to coincide with the maturation of veinlets in the young leaf. The sclereids and veinends follow a distinct combination of centripetal and centrifugal pattern of initiation and maturation.

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EXPLANATION OF PLATE XXIII

FIGS. 8, 10, and 11. Transections through mature leaves showing conspicuous polymorphic terminal sclereids, ×225 each.

FIG. 9. Growing sclereid initial cell in contact with tracheids. Note the conspicuous nucleus.

FIGS. 8–11

ON POST-EMBRYONIC STAGES OF PHYLLOPOD CRUSTACEANS, *TRIOPS* (APUS), *STREPTOCEPHALUS* AND *ESTHERIA*

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Introduction

Crustacea, whose post-embryonic stages are described in this paper, *viz.*, *Triops* (Apus) *orientalis*, *Streptocephalus dichotomus* Baird and *Estheria* are found in pools and shallow reservoirs on the table-lands of the Deccan Plateau and round about Poona. Most of these pools and ponds dry for some months during summer but the eggs of these remain in the soil. They hatch after the first showers of rain in early June. Larvæ in various stages of development can be collected during June. Adults are available up to the end of January.

The eggs of these animals are spherical and are provided with a hard coat which appears to be resistant to variations in external conditions. The

temperature of soil in Poona in summer varies from 94° F. to 124° F. (between 12 noon and 4 p.m.).

The occurrence of these phyllopods in Panchgani was noticed by several field workers. Cannon (1924) obtained a sample of dry soil containing eggs of *Estheria*, collected in the neighbourhood of Baghdad by Captain T. Buist, R.A.M.C. On placing the soil in water Cannon found that nauplii hatched out within about 24 hours and he observed that they fed on the green scum on the sides of the aquarium, on micro-organisms. He got adult *Estheria* in three weeks.

Gurney (1925) obtained specimens from Baghdad and identified the Estherid specimens as belonging to two genera. He examined about 20 specimens, two of which belonged to the genus *Leptestheria* and others to *Estheria* (=*Cyzicus* or *Eocyzicus*). He also observed that the two genera differed in rather minute details.

Gurney (1925) obtained specimens of *Apus*, some of which were from Panchgani. The Panchgani specimens were identified by him as *Apus asiaticus*. Specimens from Bulandhar in Kashmir were identified as *Apus cancriformis*. Both the Asiatic species showed many differences from the European species, *A. cancriformis*. Tiwari (1952) identified the Panchgani specimens as *Triops orientalis*, but Longhurst (1955) considered them and those collected from Rajasthan, *Apus mavliensis* as *Triops granarius*.

As early as 1885, Claus worked on the morphology, development and secondary sexual characteristics of *Branchipus* and *Artemia*. Botnariuc (1947, 1948) described the development of some phyllopods (Conchrostraca): *Cyzicus tetracerus* Kryn., *Eoleptestheria variabilis* Botn., *Lynceus andronachensis* Botn., *Leptestheria intermedia* Botn. and *Imnadia voitestii* Botn. and Orgh.

Reference to the occurrence of *Streptocephalus dichotomus* is found in the monograph by von Daday (1910). Coopey (1950) described the life-history of *Eubranchipus oregonius* and Cannon (1926) worked on the post-embryonic development of the shrimp (*Chirocephalus diaphanus*) and compared it with that of *Estheria*.

MATERIAL AND METHODS

There are various localities round about Poona where these animals are available. Dried mud or soil from Panchgani North Satara District (altitude 4296 ft. above m.s.l.) contains eggs of *Triops* and *Estheria*. Soil from a pond on the hill fortress at Panhala, Kolhapur District (altitude 2,772 ft. m.s.l.) contains eggs of *Triops*, *Estheria* and *Streptocephalus*. Soil from a pond at Sinhagad Fort, Poona District (altitude 4,320 ft. m.s.l.) contains eggs

of *Streptocephalus* and some cyprids. Adult specimens of *Streptocephalus* and *Estheria* are found in ponds in Katraj Ghat (Poona District).

Observations

Soil or dried mud from these localities was brought to the laboratory and put in small aquaria, one containing distilled water, another chlorinated tap water and the third containing muddy water from a nearby canal. Separate aquaria were used for soils from each locality. Eggs from the soil put in the aquarium containing distilled water floated for about 5–7 days, those in tap and muddy canal water hatched in about 12–24 hours. Larvæ in the muddy water hatched in about 12 hours but grew faster. Addition of bread powder and yeast did not affect relative growth, but addition of diatoms and protozoans accelerated growth. It was necessary to bubble air continuously in the aquaria.

The larvæ were fixed in Bouin's fluid at intervals and were stained with borax carmine and mounted. The following are the observations made from the whole mounts as well as from living specimens from the aquaria.

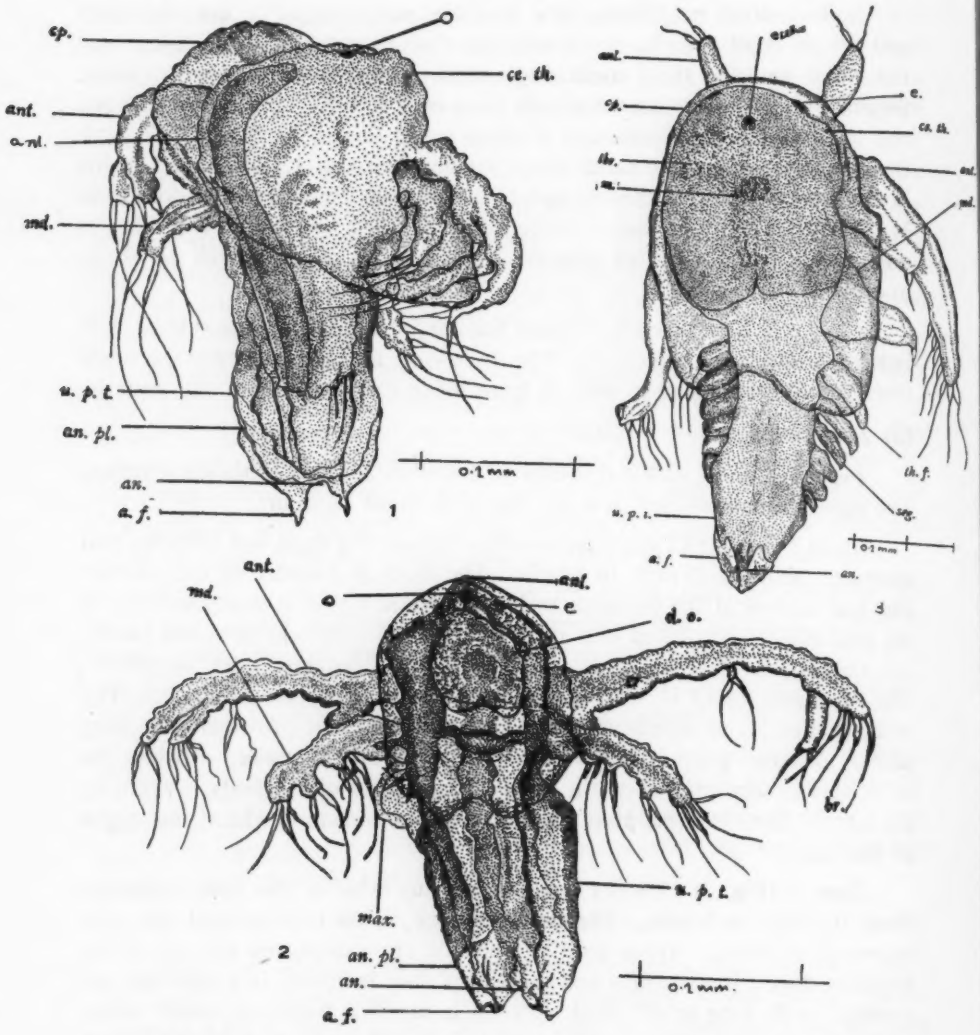
(*a*) *Triops orientalis*

Egg is spherical with a diameter of 0·2 mm. It has a chitinous covering. The eggs when immersed in water hatch in about 12 hours.

Stage 1 (Fig. 1) 12 *hours larva.*—The larva at this stage is a nauplius and measures about 0·25 mm. in length. The body is broader at the anterior end and narrow at the posterior end. The trunk region is unsegmented and the anal plate is bifurcated into anal furca (*an.f.*). The cephalothorax (*ce.th.*) has three pairs of appendages, *viz.*, antennules, antennæ and mandibles. The antennule (*anl.*) is very small and not visible beyond carapace. The antennæ (*ant.*) and mandibles (*md.*) are biramous and provided with long setaæ. Anterior portion of the body is covered with carapace. Median eye or ocellus is seen in a notch in the anterior region of the body. Ventrally the labrum (*lbr.*) is large, plate like and almost covering one-third the length of the body.

Stage 2 (Fig. 2) 17 *hours larva.*—Nauplius larva at this stage measures about 0·3 mm. in length. The cephalothorax region is broadened and also increased in length. Three pairs of cephalic appendages are seen as in the previous stage; the antennæ and mandibles have increased in length and are supplied with long setæ. Each antenna is supplied with thumb-like endopodite and a conical exopodite. Dorsal organ (Fig. 2, *d.o.*) is visible and covers most part of the cephalic region and is yellowish brownish in appearance in living specimens due to the presence of yolk material. Below the

dorsal organ, is the space of cardiac cavity (*c.c.*). The median eye is well pigmented, but the two compound eyes on either side remain unnoticed as they are less pigmented. Carapace covers half the length of the body. Anal



FIGS. 1–3. Fig. 1. *Triops orientalis*, Larva 12 hours, Lateral view. Fig. 2. *Triops orientalis*. Larva 17 hours, Dorsal view. Fig. 3. *Triops orientalis*. Larva 20 hours, Ventral view.

Magnification : Fig. 1. 18 *x*×10 *x*. Fig. 2. 18 *x*×10 *x*. Fig. 3. 10 *x*×10 *x*.

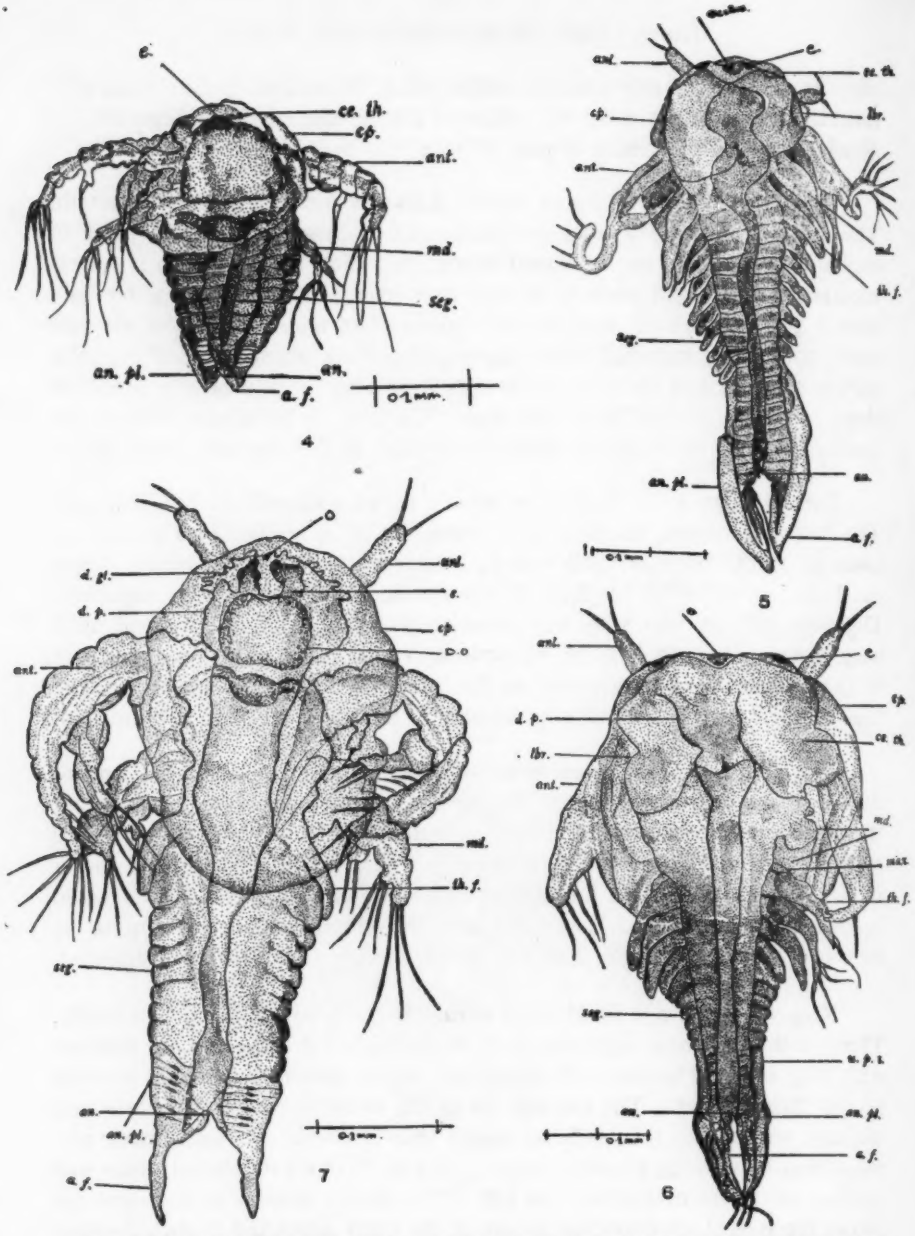
plate is very broad and ends in caudal furca. Mandible is more than one-third the length of antenna. It consists of a basal disc bearing a finger-shaped division, the palp, bearing a pair of setæ.

Stage 3 (Fig. 3) 20 *hours larva.*—Larva is about 0·4 mm. in length. The anterior end is very much increased and broadened. The three pairs of cephalic appendages are increased in size of which the antennule is unsegmented, straight and blunt and with two setæ. Below the mandibles are seen a pair of maxillæ, bud of first thoracic foot and five buds of thoracic limbs protruding followed by an unsegmented trunk portion. Median eye is very prominent and the two sessile eyes becoming big and have a brownish tinge. Labrum is still broad and large. Carapace is broadened and almost covers the first two thoracic limbs in addition to the cephalic appendages.

Stage 4 (Figs. 4 and 5) 24 *hours larva.*—Larva is about 0·55 mm. in length. The carapace covers the first three trunk limbs (Fig. 4). In Fig. 5 seven buds of trunk limbs are followed by demarcation of four segments deeply marked and followed by faint transverse lines demarcating the segments. Digestive tube is seen with two digestive pouches at its anterior end as is very clearly seen due to the transparent exoskeleton. The food particles in the alimentary canal are seen in the form of suspended particles. The first thoracic foot is seen without its endites and the anal plate is bifurcated.

Stage 5 (Fig. 6) 36 *hours larva.*—Larva is 0·65 mm. in length; appendages on the cephalothorax are the same as in previous stages, except that there is now the beginning of maxilla. Instead of the median eye two sessile eyes slightly pigmented are seen on either side gradually becoming larger in size and receding from the median eye. The caudal furca have increased in length and bear setæ. The carapace is very broad and covers almost the first two trunk segments. The first thoracic foot is seen to be segmented.

Stage 6 (Figs. 7 and 8) 48 *hours larva.*—Larva is now 0·85 mm. in length. The first three cephalic appendages show the highest growth and are supplied with long setæ. The first and second maxilla are present but usually covered by the thoracic feet. The median eye in the notch is very conspicuous and the two sessile eyes take a bean-shaped structure and are with more pigmentation than in the previous stages. In Fig. 7 (D.V.) the dorsal organ and cardiac cavity are noticeable. In Fig. 9 though the larva is of the same age shows the typical phyllopodian nature of the trunk limbs and as such deserves to be included in the next stage. The caudal styles have increased in length and end into fine setæ. Ventrally, tubular digestive gland is noticeable.

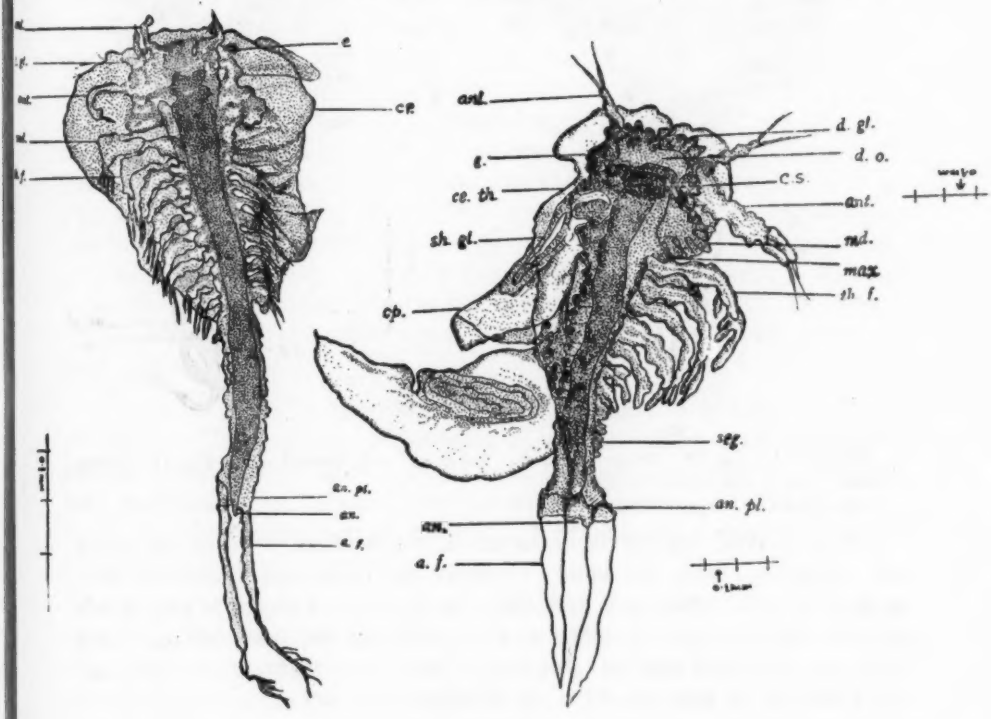


FIGS. 4–7. Fig. 4. *Triops orientalis*. Larva 24 hours, Dorsal view. Fig. 5. *Triops orientalis*. Larva 24 hours, Ventral view. Fig. 6. *Triops orientalis*. Larva 36 hours, Ventral view. Fig. 7. *Triops orientalis*. Larva 48 hours, Dorsal view.

Magnification: Fig. 4. $10x \times 10x$. Fig. 5. $10x \times 10x$. Fig. 6. $10x \times 10x$. Fig. 7. $18x \times 10x$.

Stage 7 (Fig. 9) 68 *hours larva.*—Larva is about 1·1 mm. in length. Of the first three cephalic appendages the antennule is of the same size as in the preceding stages but the antenna and mandible are smaller in size. The mandible is smaller, is a pouch-like structure with hard brownish spines on it. The first thoracic foot is with five endites and as such the animal can crawl on these feet. Six other feet are also noticeable on the thorax. Number of trunk segments is now 20. The length of the caudal styles almost equal the length of the body. Carapace has become very broad and within it the rudiments of shell gland are visible. The coils of the gland are very well seen under the carapace. The digestive gland has become very much tubular and almost fills the anterior region of the body.

Stage 8 (Fig. 10) 96 *hours larva.*—The larva is about 1·2 mm. in length. Seen ventrally the labrum is very much reduced. Antenna is still biramous;



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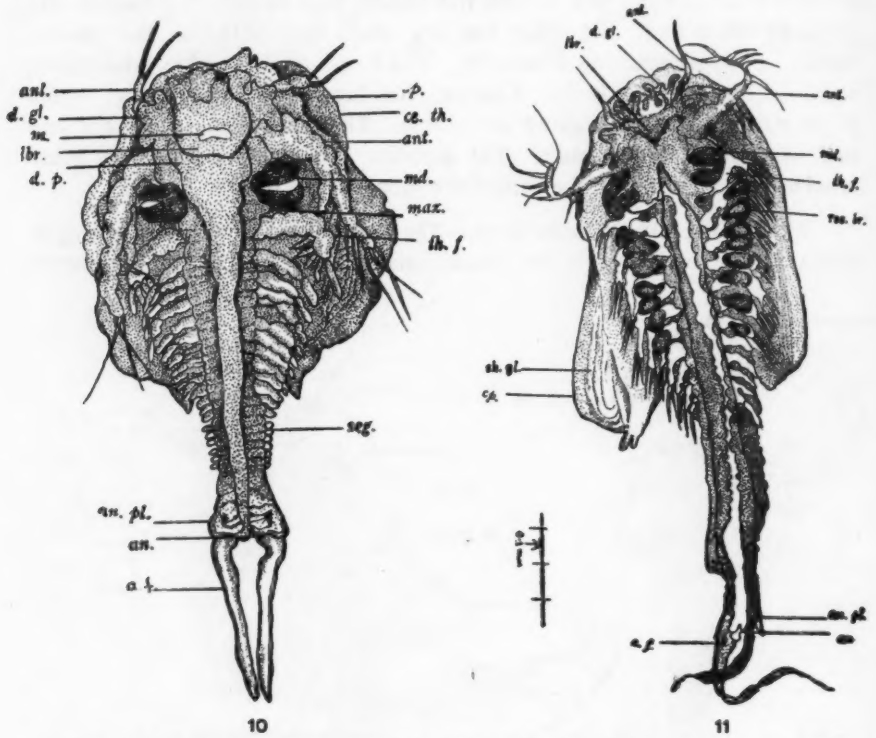
FIGS. 8–9. Fig. 8. *Triops orientalis.* Larva 48 hours, Ventral view. Fig. 9. *Triops orientalis.* Larva 68 hours, Dorsal view.

Magnification: Fig. 8. 18*x*×2·3*x*. Fig. 9. 10*x*×5*x*.

mandible is drawn out into a pouch showing further reduction. It has completely lost its setæ. The first thoracic foot with its endites is seen on either side. The carapace is now covering 11 thoracic appendages. Flabellæ are present on the first eight thoracic appendages.



FIGS. 10–11. Fig. 10. *Triops orientalis.* Larva 96 hours, Ventral view. Fig. 11. *Triops orientalis.* Larva 108 hours, Ventral view.
Magnification : Fig. 11. 10 $x \times 5$ x.

Stage 9 (Fig. 11) 108 *hours larva.*—Larva is about 1·4 mm. in length and resembles almost an adult. Ventrally the respiratory bracts are seen on first thoracic appendages and also their endites. Labrum is very much reduced and the digestive gland is very much tubular than before. Anal furca are segmented and bear fine setæ. Larva is now almost an adult and can swim on its back or crawl on its abdominal feet.

(*b*) *Streptocephalus dichotomous* Baird.

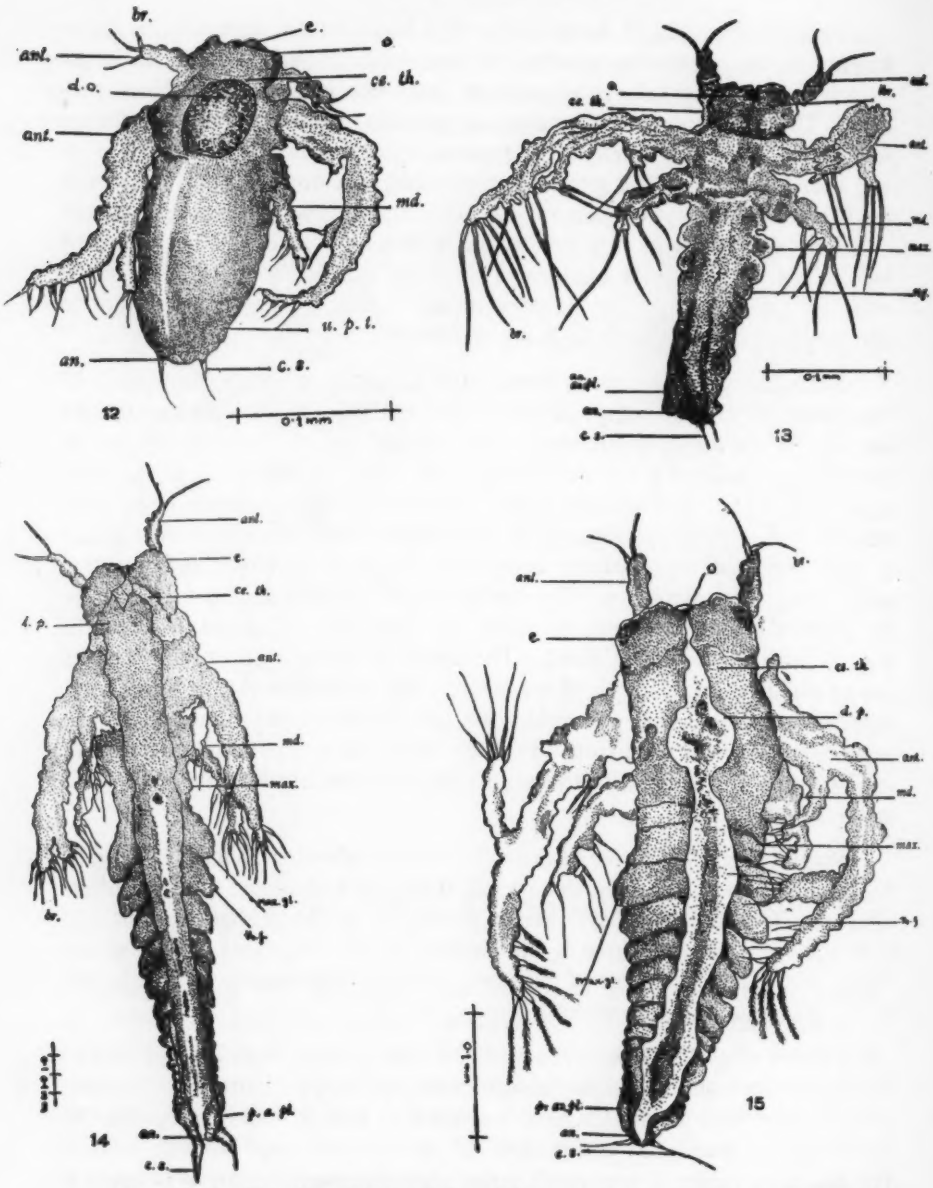
Eggs are very minute in size, about 0·2 mm. in diameter with a wrinkled sculptured coat.

Stage 1 (Fig. 12) 12 *hours larva.*—The larva at this stage is a nauplius. Its anterior end is broader than the posterior. The larva is 0·2 mm. in length with unsegmented trunk, two anal setæ and three pairs of cephalic appendages. The antennule is uniramous, unsegmented and is provided with two setæ at its anterior extremity; the antenna and mandible are both biramous and bear long setæ. The median eye (Ocellus) is a tiny red spot in between the two compound eyes which are not pigmented. Below the eyes is a dorsal organ or nuchal organ very swollen up with yolky material. Ventrally the labrum covers a considerable area of the body. It is distinguished from the other part of the body by its dark orange colour. This colour is due to the yolky material as seen in living specimens.

Stage 2 (Fig. 13) 17 *hours larva.*—The Larva at this stage measures 0·25 mm. in length, average being ·32 mm. The three pairs of cephalic appendages are seen as in the previous stage. In addition to this the rudiments of maxilla are seen below the mandibles. The trunk region is commencing to segment and ends in two anal setæ. The median eye becomes more pigmented and is very conspicuous at this stage. The compound eyes attain a slight brownish tinge. Each compound eye becomes larger and migrates away from the median eye. The dorsal organ occupies a larger portion of the cephalic region and does not show any change in the shape (not seen in Fig. 13 which is a ventral view). The antennule is very short and straight due to the increase in length of the body. The antenna is six segmented and supplied with long setæ. Ventrally the labrum covers the mouth. All the appendages seem to arise from the mid-ventral line of the body. The trunk region shows as many as five segments and also traces of caudal setæ (*c.s.*) at the posterior end.

Stage 3 (Fig. 14) 20 *hours larva.*—The larva is about 0·35 mm. in length. All the three body regions such as head, thorax and abdomen are well differentiated. The compound eyes are as prominent as the median eye but still with brownish tinge. There is an increase in the length of the maxillary region and the development of maxillæ. There is also increase in the length of the antennæ and mandibles.

Stage 4 (Fig. 15) 24 *hours larva.*—The larva is now 0·45 mm. in length. The antennule is short, straight, unsegmented and supplied with two bristles. The antennæ and mandibles have increased in length. The endopodite of the antenna is thumb-like and is supplied not only with setæ but with setules. The maxillary region is very much larger since the maxillary gland (excretory gland) is very much developed in the larval stages. The bud of first thoracic limb is also noticeable. In the trunk region buds of trunk appendages

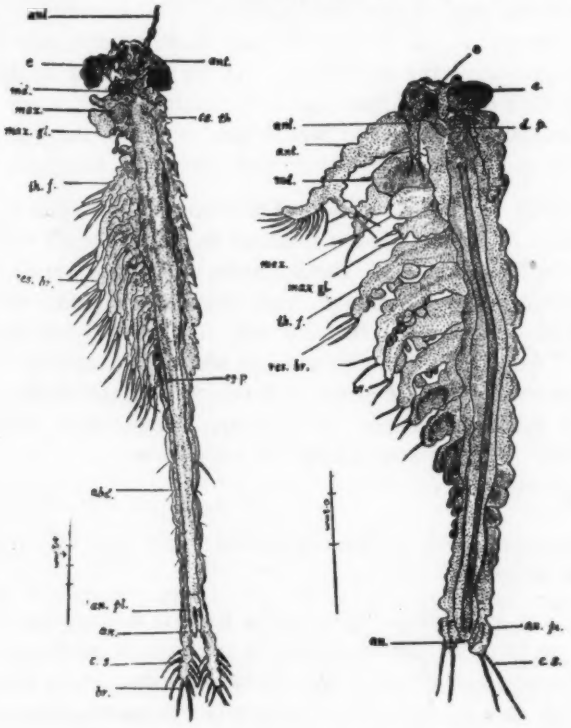


FIGS. 12–15. Fig. 12. *Streptocephalus dichotomus*. Larva 12 hours, Dorsal view. Fig. 13. *Streptocephalus dichotomus*. Larva 17 hours, Ventral view. Fig. 14. *Streptocephalus dichotomus*. Larva 20 hours, Dorsal view. Fig. 15. *Streptocephalus dichotomus*. Larva 24 hours, Dorsal view.

Magnification : Fig. 12. 18 $x \times 10$ x. Fig. 13. 15 $x \times 10$ x. 14. 10 $x \times 10x$. Fig. 15. 10 $x \times 12{\cdot}5$ x.

numbering five protrude out followed by markings of two segments and finally there is an unsegmented portion. The alimentary canal is seen with two digestive pouches below the transparent exoskeleten and the food is seen in the form of suspended particles. The compound eyes become pigmented at their anterior end.

Stage 5 (Fig. 16) 48 *hours larva.*—The larva is about 1·1 mm. in length. The compound eyes are elevated above the cephalic region and are borne on stalks. The antennæ and mandibles are reduced in size. In front of the eyes the frontal organs are also noticeable. Mandible is now in the form of a pouch. Maxillary gland is well developed below the mandibles. Eleven pairs of thoracic appendages are seen, out of which the first four are seen with endites and the remaining are in the form of protuberances. The bracts of the first four limbs are clearly formed. The flabellæ are also dis-



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FIGS. 16–17. Fig. 16. *Streptocephalus dichotomus.* Larva 48 hours, Dorsolateral view. Fig. 17. *Streptocephalus dichotomus.* Larva 96 hours (female), Lateral view.
Magnification: Fig. 16. 10 *x* × 10 *x*. Fig. 17. 10 *x* × 3·2 *x*.

tinctly formed and show the clear demarcation from the last endite at one end and the bract at the other. The first thoracic limb is smaller in size than the second. The thoracic and the abdominal region are more clearly marked than in the previous stages. The abdominal region is without any appendages. The caudal furca with caudal setæ is noticeable. The head region has increased in size and breadth. The antennules and antennæ become more ventral in position. In the case of antenna there is a constriction at the basal region and this is probably due to the bending of the antenna ventrally, which is slowly attained during the process of reorientation.

Stage 6 (Fig. 17) 96 *hours larva.*—The larva is 3·3 mm. in length. The appendages of the thoracic region very well developed and are followed by several abdominal segments without appendages. The 1st eight appendages of the thorax have well marked endites, flabellæ and bracts. The first limb appears distinctly smaller than the 2nd limb. The antenna at this stage is completely rotated on the ventral side and is very much reduced and knob-like thereby indicating the female sex. At the same time at the " Analage " of 11th and 12th thoracic appendage a thickening in the form of a paired rounded elevation is developed which goes to form the egg-pouch of the female. The caudal furcæ are elongated, plate-like and with setæ.

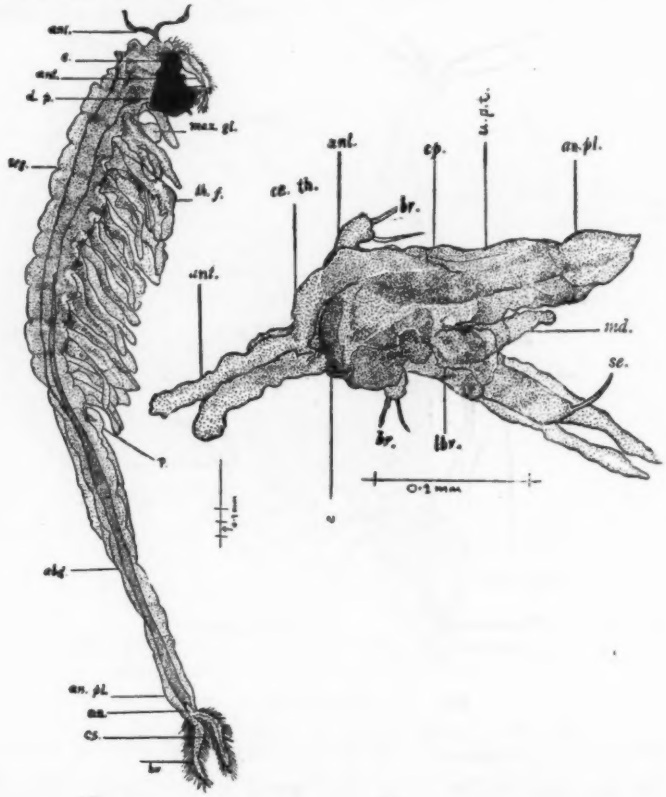
Stage 7 (Fig. 18) 10*th day larva.*—The larva is almost adult now, measuring about 5·1 mm. in length. The abdominal segments are all well marked and the last is the longest of all. Development of all thoracic limbs except the last is complete. The mandibular and maxillary regions are very much reduced. The antenna has become very much plumose and prehensile. At the very " Analage " of the 1st and 2nd abdominal segment a raised pouch in which the male retractile penis is developing is noticeable. The frontal organs have become plumose and sensory. The caudal furcæ have five setæ on them. The larva is almost an adult now.

(*c*) *Estheria*

Eggs resemble those of *Streptocephalus*; they are very minute in size, 0·2 mm. in diameter.

Stage 1 (Fig. 19) 17 *hours larva.*—The larva is a nauplius, 0·25 mm. in length with no trace of segmentation in the post-mandibular region. The carapace is bivalve and almost covers the entire body. Three pairs of cephalic appendages are seen of which the antennule is uniramous, unsegmented and is supplied with two setæ. The antenna and mandible are biramous. Ventrally, labrum is large and covers one-third the length of the body. Median eye is pigmented and is found in the anterior notch in the cephalic region.

Labral glands are very prominent at the margin of the labral plate. The anal plate is not bifurcated.

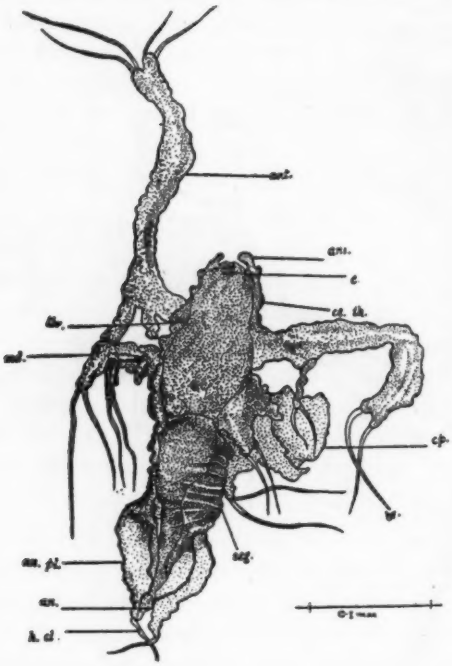


FIGS. 18–19. Fig. 18. *Strepto-cephalus dichotomus.* Larva 10th day, Dorsolateral view (unale). Fig. 19. *Estheria.* Larva 17 hours, Lateral view.
Magnification: Fig. 18· 8 *x*×2·3 *x*. Fig. 19. 18 *x*×10 *x*.

Stage 2 (Fig. 20), 20 *hours larva.*—Larva is now a metanauplius, 0·3 mm. in length. About 9 segments are seen in the post-mandibular region. Carapace is folded on one side of the cephalic appendages, the antennule is stumpy and blunt. The antenna has increased in size and is supplied with very long setæ. The mandible is also biramous and with long setæ. The anal plate is bifurcated into hooked claws. Ventrally the labrum is a long plate covering one-third the portion of the body.

Stage 3 (Fig. 21) 48 *hours larva.*—Larva is now 0·65 mm. in length. In front of the eyes are sensory papillæ. Each thoracic segment bears a curved

spine on its dorsal side. The carapace is well marked bearing very small spines at regular intervals at its margin and covers first eight thoracic appendages.



20

FIG. 20. *Estheria.* Larva 20 hours. Ventral view.
Magnification: Fig. 20. 18 $x \times 10$ x.

Within the carapace rudimentary shell gland is noticeable. The digestive tube is seen with the digestive pouches and is encircled by tubular digestive gland. Seven trunk appendages with rudiments of buds of two more trunk appendages are noticeable. The caudal hooked claws are longer and are supplied with spines at their extremity.

Stage 4 (Fig. 22) 68 *hours larva.*—The larva is about 1·4 mm. in length. The antenna and mandible are reduced in size. Mandible is reduced to a pouchlike structure. Thoracic appendages are with bracts and the typical phyllopodian nature of limbs is noticeable in first eight thoracic limbs. Four buds of thoracic appendages are present. Two sessile prominent black eyes are seen on either side of the antennule.

Stage 5 (Fig. 23) 96 *hours larva.*—Larva is 1·3 mm. in length. The shell gland, which is the excretory organ, is well developed within the carapace.

Six endites are seen to each thoracic appendage. Number of hind segments is increased to 9. The carapace is seen with " lines of growth " on it. Larva almost looks like an adult *Estheria.*

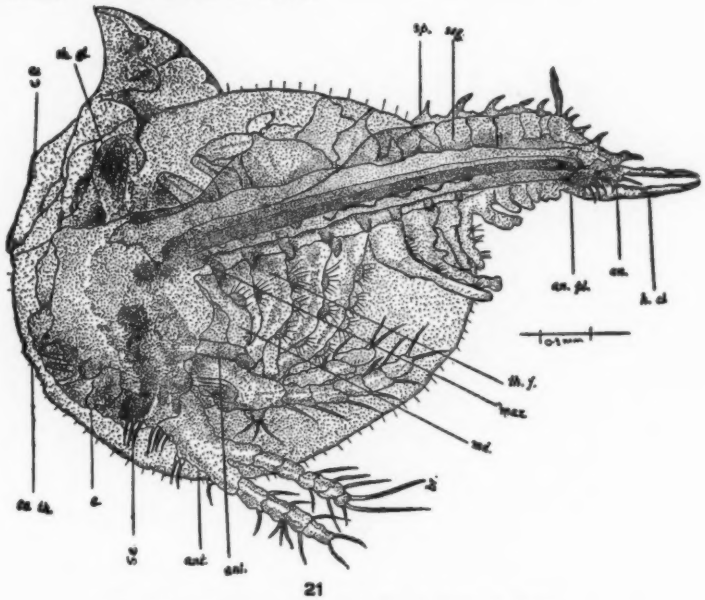


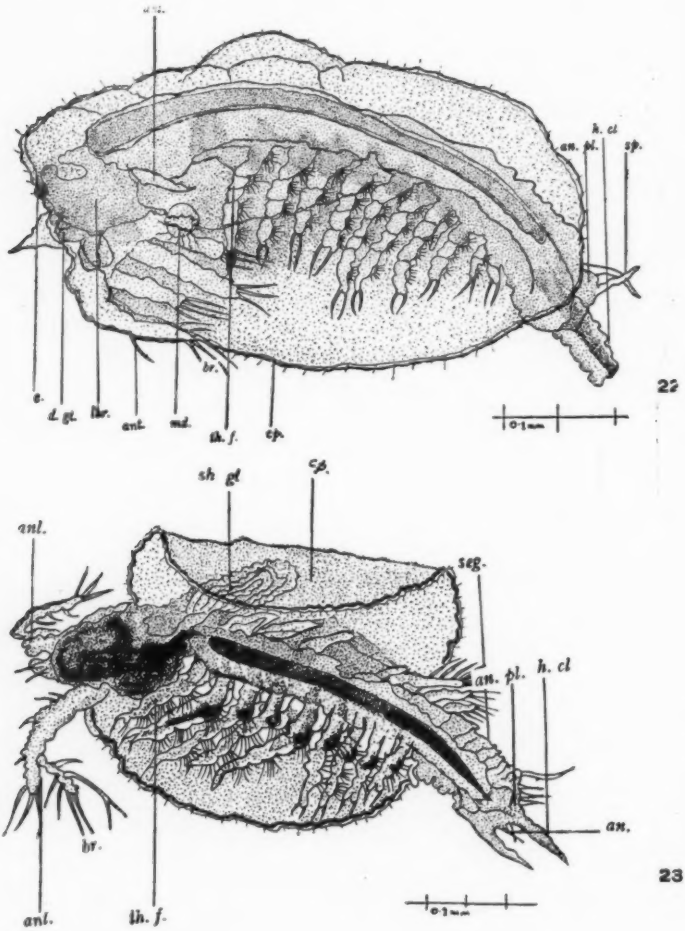
FIG. 21. *Estheria.* Larva 48 hours, Lateral view.
Magnification : Fig. 21. 10 *x* × 10 *x*.

DISCUSSION

The newly hatched larva is either a true nauplius without any segmentation in the trunk region, but possessing a median nauplius eye in the notch in the anterior part of head (seen ventrally) and three pairs of cephalic appendages or a metanauplius showing segmentation in the post-mandibular region. They show phototrophic movements and tend to move to the top of the aquarium to feed on the scum deposited on its sides. Scum when examined is full of protozoa, diatoms and algæ. Longhurst (1955), Hall (1933), Cannon (1924 and 1926) obtained larvæ of various phyllopods from mud samples by placing them in tanks of clean water.

The time taken for hatching after the immersion of mud containing eggs is about 12 hours. This period is likely to vary. If only a small quantity of mud is placed in a deep petri-dish, the larvæ hatch in 12 hours but in a large aquarium with a larger quantity of mud, the larvæ hatch in about 24–48 hours. Most workers have obtained nauplius larvæ in 24–48 hours.

The larvæ also hatch out more quickly in hot season than in winter. All these observations are made at room temperature which is different in both the seasons.



FIGS. 22–23. Fig. 22. *Estheria.* Larva 72 hours, Lateral view. Fig. 23. *Estheria.* Larva 96 hours, Lateral view.
Magnification : Fig. 22. 10 $x \times 10$ x. Fig. 23. 10 $x \times 5$ x.

The larvæ of the three crustaceans can easily be distinguished from each other immediately at hatching. The larvæ of *Triops* have a carapace covering

the anterior part of the body, bifurcated anal plate, three pairs of cephalic appendages and a median pigmented nauplius eye seen ventrally. The larva of *Streptocephalus* has no carapace, no segmentation in the post-mandibular region but possesses three pairs of cephalic appendages, two anal setæ, a prominent sessile median eye in the notch in the anterior part of the head (seen ventrally). The larva of *Estheria* has carapace in the form of a bivalve shell without any striation. The shell covers almost the entire body. The larva possesses three pairs of cephalic appendages, a median nauplius eye (seen ventrally) with pigmentation and a large labrum overlapping one-third the length of the body. In the metanauplius stage of *Estheria* the trunk shows segmentation and an anal plate without paired hooked claws. The shell does not cover the whole body but keeps the head and telson free. These observations are in agreement with those of Botnariuc (1947) on the phyllopod conchostraca (*Leptestheria intermedia* Botn.), *Eoleptestheria variabilis* Botn. and *Imnadia viotestii* Botn. (1948).

Carapace.—Streptocephalus has no carapace at all. The carapace of *Estheria* is not moulted during ecdysis but seems to be retained and shows " Lines of growth " and from these lines one can conclude the age of the animal (Lankester). In the case of *Triops*, moulting takes place together with the carapace. This was well noticed while observing the various larval stages in the cultures. Longhurst (1955) while working on the individuals of *Triops cancriformis* and *T. granarius* noticed that the ratio of the carapace length to the total length of body remained the same throughout the growth. The basic variation is seen in the size of the carapace relative to the total length of the body. While observing the various larval stages of *Triops*, it is noticed that when the carapace is small in size, it is rounded in outline and is stronger in structure but when it is broad, it is flat and less strong.

Segmentation and appendages.—External appearance of segmentation in all these animals is noticeable 20 hours after hatching when along with trunk segments, 4–5 buds of appendages of the trunk are seen to protrude. This is the condition in *Triops* and *Estheria*. But in *Streptocephalus* buds of trunk appendages commence 24 hours after hatching though the cultures are at the same room temperature. The first thoracic appendage with its endites is seen in the larvæ of *Streptocephalus* and *Estheria*, 36–48 hours after hatching and the distal endite of this appendage develops into a respiratory bract. But in the case of *Triops* the distal endite of the first thoracic appendage forms the respiratory bract as late as 4th day after hatching. Lankester (1909) stated that the epipodites are richly supplied with blood and he called them " Branchiæ ".

Linder (1952) working on the North American Notostraca elucidated the relationship between the number of segments, the number of appendages and the number of posterior apodous segments. He concludes that the number of appendages is the result of two quite separate processes: the production of segments and appendages in the larvæ proceed at different rates. The boundaries of the appendages bearing segments are not complete ventrally; no correlation could be found between the number of appendages and the segments bearing them. The appendages may have no relation to the length of segment.

The three cephalic appendages, *viz.*, the antennules, antennæ and mandibles show interesting variations in the three phyllopods studied. The antennule is almost constant in size. It is uniramous, unsegmented and supplied with sensitive bristles at its upper extremity. The antennæ and the mandibles are typically biramous and supplied with long setæ in early larval stages. They increase in length with age; maximum length is attained in larvæ 36–48 hours old. Later these appendages undergo reduction. The antenna becomes small in size except in *Streptocephalus* male where it is plumose and, by the time metamorphosis is complete, almost vestigial. The mandible loses its biramous character and develops into a cup-shaped structure with spines. The first trunk appendage by this time is fully developed and is provided with endites and it functions as a swimming and crawling appendage. As early as 1886, Claus observed that the lobed leaf-like appendages suggested the primitive form of crustacean appendage inasmuch as the branchiopod limb resembles the early phylogenetic stage before the evolution of the biramous type.

Eyes.—The median pigmented nauplius eye seen in early larval stages (12 and 20 hours) is seen in a notch in the anterior part of the head. In the case of *Streptocephalus* there is a median eye in the nauplius stage and it persists up to 36 hours and then paired sessile eyes appear which are pigmented. Slowly, in larvæ 48 hours old the eyes are borne on stalks. In the case of *Triops* and *Estheria*, there is a median eye with pigmentation and the two sessile eyes on either side of the dorsal organ in larvæ 36 hours old, but pigmentation is less marked. Seventy-two hours old larvæ of these animals show two sessile eyes on either side of the dorsal organ with dark pigmentation.

Maxillary gland.—The maxillary gland of *Streptocephalus* which is found below the 2nd maxilla makes its first appearance in a 36 hours old larva. It becomes conspicuous in 48–68 hours and more prominent in 72 hours. The shell gland in the case of *Triops* and *Estheria* makes its appearance in 48 hours old larva and is very well developed by the 4th day. The digestive

gland in all these animals is first seen at the 48 hours stage and becomes much more tubular during 72–96 hours stages and thereafter is very prominent in the animal.

Sex differentiation.—The larvæ develop into adults, but the adult forms survive under laboratory conditions just for one and a half month. Sex differentiation is particularly well noticed in *Streptocephalus*. As early as the 10th day in a larva with 11 pairs of appendages and with the appearance of 12th segment, the "Analage" of either a double penis or egg-pouch could be seen. The penis is retractile and is beset with curved spines when everted. The egg-pouch of the female has a red patch on its ventral surface and bears an opening controlled by a sphinchter at its distal end. The antenna of the female is plate-like. The male has a twisted plumose prehensile antenna used as " sexual clasper " (Lankester, 1909).

Some of the females of *Streptocephalus* were isolated and kept in another aquarium full of water and with ordinary dried mud. These laid eggs which were without chitinous covering and they soon decomposed and did not germinate. It appears, therefore, there is no parthenogenesis in these animals.

In *Estheria*, during the third week of development the male develops hooked claws to the first two endites of the first thoracic appendage. These claws are also used as sexual claspers with which the female is held. The female shows continuous rhythmic movement of the abdomen. The male and female are seen attached to each other lengthwise one behind the other, for many days. Later, they bury themselves in the mud with periodical spurting movements in the aquarium. After copulation, the animals become whitish in colour and look very sluggish in their activity. The whole life-cycle of the animal is completed within 40 days. Eggs extruded from the egg-pouch were placed on a filter-paper and the paper was kept to dry for about 24 hours. On immersing the filter-paper in water, larvæ hatched in 4–5 days or even as early as 48 hours. It is a remarkable fact that the eggs laid in an aquarium did not germinate in the same aquarium, but if it was emptied, dried for a week and then refilled, the eggs germinated in about 48 hours. A period of desiccation appears to be necessary for the eggs to germinate under natural or normal conditions. Eggs do not hatch in pools or ponds where they are laid unless the pool or pond dries and new showers fall in the next monsoon. Heath (1924) while summarising his observations on the Indian Anostraca remarks that both specimens of *Branchinecta orientalis* and *Branchipodopsis affinis* have eggs requiring freezing and certainly that of *Branchipus* and of the *Streptocephali* require drying.

Evidence of sex differentiation is not so readily obtainable in *T. granarius* except in the second week of development when males look pale and brownish and the females deep green. The males possess long caudal styles. The females possess a broad carapace beneath which an egg-pouch is formed to the 11th appendage.

All the three animals can be kept in an aquarium for about 2 months. In about 40 days the life-history of the animals is completed. After copulation the animals become senile and die. The conditions in the aquarium retard their growth.

Movements to light and food.—Cannon (1924) described the larvæ of *Estheria* as showing phototropic movements and it was noticed during the course of the work, that during night if a light from a torch was thrown on the aquarium, the larvæ showed a very spurting movement. During the day they now and then congregated at the surface on the top of the aquarium towards the bright side and fed on protozoa and diatoms.

ACKNOWLEDGEMENTS

I am grateful to Dr. Karekar for supplying samples of mud to Prof. D. D. Samarth for training me in the technique and to Dr. M. A. Moghe for guidance.

SUMMARY

1. Eggs from mud take 12–48 hours to hatch.

2. The newly hatched larva in *Estheria* is a nauplius or a metanauplius.

3. It shows phototrophic movements and feeds on diatoms and minute micro-organisms. Scum when examined was full of diatoms, algæ and protozoans.

4. Three pairs of cephalic appendages show maximum growth in length within 36–48 hours after hatching but after 72 hours progressive reduction takes place so that the antenna becomes almost vestigial except in male *Streptocephalus* and the mandible changes into a cup-shaped structure with spines on it, the antennule, however, persists.

5. Segmentation of trunk region increases progressively leaving in all cases a posterior unsegmented region.

6. Buds of appendages on the trunk segments increase in regular anterio-posterior order.

7. During the formation of appendages the distal endite of the thoracic appendage in the case of *Streptocephalus* and *Estheria* develops into a respi-

ratory bract early, 48 hours after hatching, but in the case of *Triops* as late as on the 4th day.

8. In *Streptocephalus* sexual difference is noticeable during 2nd week of larval life, when 11 pairs of appendages are developed, either in the form of a penis or an egg-pouch. In male antennæ become plumose and rephensile. In male *Estheria* the first two endites of the first thoracic appendage develop hooked claws on them.

9. The caudal appendages show variations in the three larvæ. It is long, rod-like, segmented and supplied with bristles in *Triops*; plate-like and supplied with very fine bristles in *Streptocephalus* and in the form of hooked claws in *Estheria*.

10. Median sessile eye degenerates and the two compound eyes on either side remain sessile in *Triops* and *Estheria* but become stalked in *Streptocephalus*.

11. The eggs extruded in an aquarium do not germinate in the same aquarium but require a period of desiccation.

12. Females when isolated produce naked eggs (without shell) which decompose after a time thereby showing that parthenogenesis does not take place. The naked eggs dried on a filter-paper and immersed in water do not germinate.

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ABBREVIATIONS USED IN TEXT-FIGURES

abd., abdomen; *a.f.*, anal furca; *an.*, anus; *an.pl.*, anal plate; *anl.*, antennule. *br.*, bristel; *ce.th.*, cephalothorax; *cp.*, carapace; *c.c.*, cardiac cavity; *c.s.*, caudal seta; *d.o.*, dorsal organ; *d.gl.*, digestive gland; *d.p.*, digestive pouch; *eg.p.*, egg-pouch; *e.*, eye; *h.cl.*, hooked claws; *l.br.*, labrum; *m.*, mouth; *md.*, mandible; *max.*, maxilla; *max.gl.*, maxillary gland; *o.*, ocellus (median eye); *p.*, pouch to denote male sex; *res.br.*, respiratory bracts; *se.*, seta; *seg.*, segment; *sp.*, spine; *u.p.t.*, unsegmented portion of the trunk.

ISO-CHROMOSOMES AND THE ORIGIN OF TRIPLOIDY IN HYBRIDS BETWEEN OLD AND NEW WORLD SPECIES OF *PHILADELPHUS*

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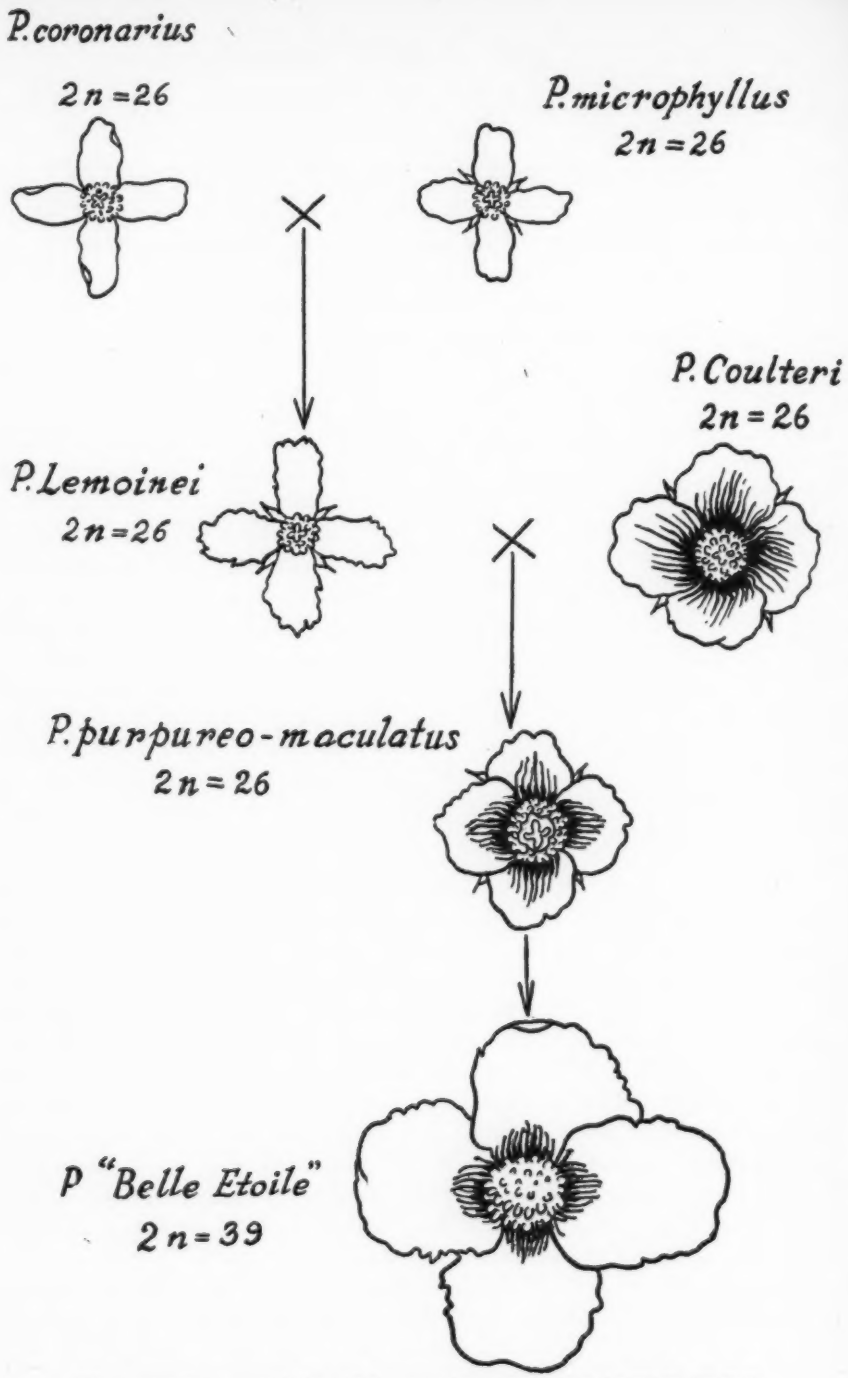
The genus *Philadelphus* comprising about 40 species of deciduous shrubs is found in isolated regions in North America, East Europe, Caucasus and East Asia to Himalayas. The fossil history of *Philadelphus* goes as far back as Eocene times when the genus had a continuous distribution in the Northern Hemisphere. With the rise of the Rocky Mountains, the distribution of *Philadelphus* in the New World became discontinuous and the result has been the evolution of distinct eastern and western geographical races in North America. The West American species, which are more closely related to the fossil types, have inflorescences which are racemose panicles. The Ice Age in America saw the migration of *Philadelphus* southwards as far as Mexico, and in these regions have evolved, desert forms like the *Microphylli*, which have small leaves and scented flowers which are usually solitary. At least four distinct geographical races have evolved in America as a result of isolation (Rehder, 1940).

In Asia a north-south differentiation of *Philadelphus* occurred as a result of the Himalayan uplift. To the North Asian group, the *Coronarii* series of Rehder, also belong the single European species *P. coronarius* and the related *P. caucasicus* which survived the last Ice Age by finding shelter in the mountains of South Europe and Caucasus respectively. The Indian species of *Philadelphus*, *P. tomentosus* and *P. nepalensis* of the Himalayas, are closely related to these, and link the *Coronarii* of Europe with their Siberian, Manchurian and Japanese counterparts. The second group of Asian *Philadelphus*, the *Sericanthe*, are found in Central and West China. They resemble *Deutzia*, the genus most closely related to *Philadelphus* and have the same basic number of 13 chromosomes. While high polyploidy occurs in *Deutzia* (Sax, 1931), *Philadelphus* has remained diploid ($2n = 26$) nature since Eocene time (Bangham, 1929).

In an earlier paper (E. K. Janaki Ammal, 1951) I have traced the history of *Philadelphus* in cultivation and reported on the occurrence of triploids



Diagrammatic representation of the parentage of the triploid "Belle Etoile".

amongst the progeny of garden hybrids between species widely separated in nature.

These triploids ($2n = 39$) which had much larger flowers than diploids were first found in the progeny of the tri-specific hybrid *P. purpureo-maculatus*. The grandparents of this hybrid were the European species *P. coronarius* and the fragrant Arizonian species *P. microphyllus*. The resulting hybrid *P. lemoinei* was crossed with the Mexican species *P. coulteri*. This gave the sweet scented and purple tinted *P. purpureo-maculatus*. Both fragrance and size have been enhanced as a result of triploidy in the progeny of this garden favourite. The best known of them is "Belle Etoile" (*see* diagrammatic sketch).

The production of unreduced gametes which is the main cause of aberrant triploid seedlings in cultivated plants has been shown to be due to errors in meiosis. Triploids are not only of immediate value to horticulture and agriculture as being more decorative or vegetatively productive plants, but they also become the potentials of hexaploids by chromosome doubling. Hexaploids are increasingly found in regions where the distribution of diploid species overlap. This is a common occurrence in the case of *Magnolia*, *Camellia* and *Rhododendron* (E. K. J., 1950, 1952 *a* and 1952 *b*). The chromosome mechanism that is responsible for the production of triploids in garden hybrids between species widely separated in nature as in *Philadelphus* is of interest for understanding what happens in nature when diploid populations overlap as a result of migration or due to changes in the physiography of land masses.

The Chromosome Morphology in *Philadelphus*

For the study of chromosomes, flower-buds were fixed in acetic alcohol 1:3 and stained with acetocarmine. The chromosome complement of *Philadelphus* was fairly uniform in all the 29 species I examined. It was found to be composed of 13 pairs of chromosomes. The chromosome morphology is best studied during mitosis in pollen grains (Fig. 1). Of the 13 chromosomes, composing the haploid complement, 4 are small, 8 medium and 1 long. The single long chromosome and 2 of the small chromosomes have medium constrictions while in the remaining 10 it is subterminal. The haploid formulæ may be represented as:

$$1\,L_m + 8\,M_{st} + 2\,S_{st} + 2\,S_m.$$

The large L chromosome stands out from the rest by being more heterochromatic than the others.

CHROMOSOME BEHAVIOUR IN *Philadelphus*

Thirteen bivalents are formed regularly during meiosis in all the species studied (Figs. 2 and 3). Of these five have 2 chiasmata and eight have 1 chiasma only, the chiasmata frequency being 1·38.

In spite of regular pairing pollen sterility was found to be fairly high in some of the species studied (*see* Table I).

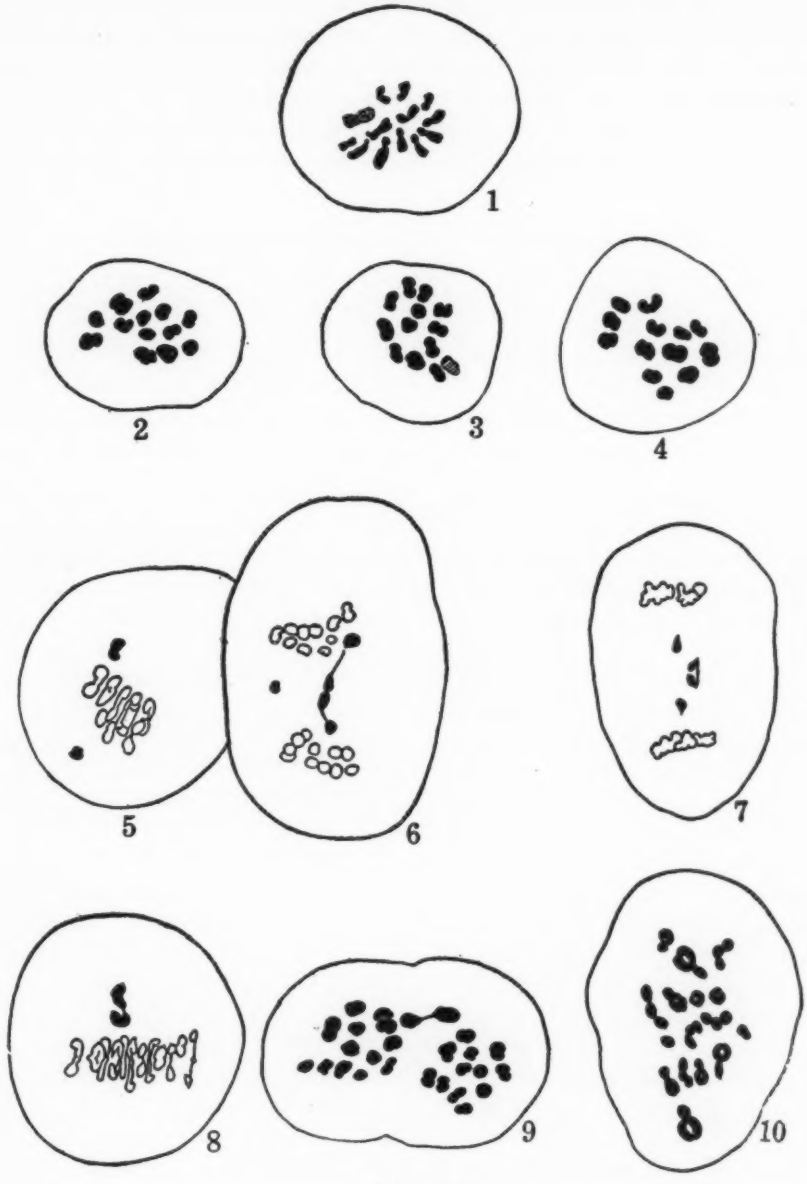
An examination of pollen mother cells in the parent species showed that the behaviour of the single large pair of chromosomes differed from the rest of the complement at meiosis. It did not congress on the plate simultaneously and behaved as if it had a defective chromosome. This behaviour was accentuated in the hybrids. Figs. 5–8 show the behaviour of the L chromosome in two hybrids *P. zeyheri* and *P. lemoinei*. In *P. lemoinei* the two large chromosomes failed to pair quite often and the formation of chiasma between the two arms of the univalent chromosomes was often noted (Fig. 5) showing that this chromosome is an *iso-chromosome*. Misdivision was fairly common and clearly seen during anaphases (Figs. 6–7).

The behaviour of the iso-chromosome differed in the different species and could be correlated with the pollen sterility. Thus in some of the species like *P. grandiflorus* of America and *P. tomentosa* of the Himalayas, the pollen sterility was almost as great as in interspecific hybrids. In general, the iso-chromosomes of the *Coronarii* and *Speciosii* groups were found to be more inert than in the other races.

The percentage of pollen fertility of 9 species and 3 hybrids is given in Table I.

TABLE I

	%
P. microphyllus	95
P. californicus	94
P. argyrocalyx	93·6
P. coulteri	92·4
P. Sakamuri	80
P. coronarius	62
P. mexicana	62
P. tomentosa	54
P. grandiflorus	49
Hybrids	
P. zeyheri (*P. coronarius* × *P. grandiflorus*)	50
P. lemoinei (*P. coronarius* × *P. microphyllus*)	45
P. purpureo-maculatus (*P. lemoinei* × *P. coulteri*)	41



FIGS. 1–10.

In the *Tri-specific hybrid Philadelphus purpureo-maculatus* (*P. lemoinei* × *P. coulteri*), the chiasma frequency was reduced to 1·0. Out of 192 pollen grains of *P. purpureo-maculatus* examined, 20 were found to be binucleate and 1 was four-nucleate. Pollen fertility was only 41%. Fig. 9 shows the formation of binucleate pollen grains by the non-separation of 2 iso-chromosomes during anaphase. When these function the result is a triploid.

CHROMOSOME BEHAVIOUR IN TRIPLOID *Philadelphus*

Upto 11 trivalents were found in pollen mother cells of the triploid "Belle Etoile" (Fig. 10); Of the remaining chromosomes either one or two are bivalents, the rest being univalents. Iso-chromosomes were generally found at the periphery of the cell forming a trivalent, or associated as a bivalent and univalent. The pollen fertility in the triploid was reduced to 24%.

While geographical isolation in *Philadelphus* has not appreciably affected pairing between the 12 pairs of chromosomes in hybrids, it has a distinct effect on the pairing of iso-chromosomes. It seems from these studies that heterozygosity is more pronounced in the iso-chromosome than in the ordinary chromosome.

The persistence of an iso-chromosome in the chromosome complex of the genus *Philadelphus* in which the species are so many and so widely separated in nature is interesting when compared to the function of iso-chromosome in other plants. Permanent iso-chromosomes have been noted in other genera of plants, notably in the *Solanaceæ* (E. K. J., unpublished). In *Nicandra* which is a monotypic genus and probably a relic species, Darlington and E. K. J. (1945) found that plants with single iso-chromosome showed delayed germination. The evolutionary bearing of iso-chromosome of *Philadelphus* could only be studied by tracing its behaviour and persistence in successive generation of diploids. This work had to be postponed for geographical reasons. However, Sampson (1955) has studied the progeny of one of the triploids—Bicolore, and according to his findings the chromosome numbers of the progeny are nearer diploids than triploids. He also finds that the distribution of extra chromosome is not at random.

SUMMARY

1. All *Philadelphus* species in nature are diploids $2n = 26$.

2. The chromosome complement of species examined was found to be fairly uniform in morphology and could be represented by the haploid formulæ.

$$1\,L_m + 8\,M_{st} + 2\,S_{st} + 2\,S_{sm}.$$

3. The L chromosome in *Philadelphus* was found to be an iso-chromosome with a weak centromere.

4. While geographical isolation in *Philadelphus* has not appreciably affected pairing between the 12 pairs of chromosomes in hybrids, it has a distinct effect on the pairing of iso-chromosomes.

5. Pollen fertility of species and hybrids is related to the behaviour of iso-chromosome.

6. Triploidy in interspecific hybrids is due to the formation of unreduced gametes by non-separation of iso-chromosomes at meiosis.

ACKNOWLEDGEMENT

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EXPLANATION OF TEXT-FIGURES

FIG. 1. Somatic metaphase in pollen grain division of *P. microphyllus* showing 13 chromosomes with iso-chromosome shaded.

FIGS. 2–4. Metaphase I in pollen mother cells of *P. coronarius*, *P. lemoinei* and *P. microphyllus* showing 13 bivalents.

FIG. 5. Metaphase I of *P. lemoinei* showing 2 iso-chromosomes outside the plate, one paired on itself.

FIG. 6. Anaphase I of *P. lemoinei* showing misdivision of paired iso-chromosomes.

FIG. 7. Anaphase of *P. lemoinei* showing misdivision in unpaired iso-chromosome.

FIG. 8. Metaphase I in *P. zeyheri* showing non-congression of paired iso-chromosome.

FIG. 9. The formation of binucleate pollen grain in *P. purpureo-maculatus* by non-disjunction of iso-chromosome.

FIG. 10. Metaphase I in "Belle Etoile," the triploid hybrid.

All the figures were drawn at the magnification ×1,800.

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STUDIES IN SAMPLING TECHNIQUE

IX. Estimation of Stalk-Basis Incidence of Sugarcane Borers in a Field

By R. C. Acharya, S. K. Prasad and K. L. Khanna, F.A.Sc.
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Received April 22, 1958

I. Introduction

Borer activity in a sugarcane field may be defined as percentage of stalks affected and also as percentage of joints or length of canes damaged. The stalk-basis incidence measures the extent of infestation, while length- and joint-basis incidences are measures of its intensity. In this paper the efficiency of a number of sampling designs has been examined for estimation of stalk-basis incidences of the three kinds of sugarcane borers (top, stem and root) with a pre-assigned degree of accuracy.

II. Review of Literature

Fleming and Baker[1] have developed a method for estimating the population of larvæ of the Japanese beetle and noticed that it was possible to estimate a dense population more accurately than a sparse population. Marshall[2] has evolved a sampling design for the estimation of insect population with special reference to American boll-worm (*Helothis obsoleta* Fabr.) and observed that variation between rows was greater than within rows. In a study of the distribution of the population of cotton boll-worm, Pradhan[3] also obtained similar results. Beal,[4] while examining the different sampling designs for estimating insect population in a field, found that " marked reduction in the area which must be examined to secure a given degree of accuracy in the estimate of population could be secured, first, by stratification and secondly, by making the number of sampling units examined in a given stratum proportional to the standard deviation for the sampling units in that stratum". No studies have been done in this country so far except those of Khanna *et al.*,[5–8] in which the authors have evolved a detailed sampling technique based on multi-stage design for estimating field incidences of sugarcane whitefly, pyrilla, mite and borers. The paper of Khanna *et al.* on borer incidence has derived the sampling technique and sample size for obtaining estimate of incidence on length basis pooled for the three kinds of borers.

III. THEORETICAL BASIS

The ultimate sampling units in this study are the sugarcane stalks in a field which are classified into either of the two classes, *viz.*, healthy or affected and our aim is to find out minimum sample size for an estimate of proportion of stalks affected in the field with a desired degree of accuracy. The

FIG. 1. Showing the distribution of borer infestation in the field.

expressions for sample estimates of population proportions and their variances in respect of the sampling designs examined in the paper are given below.

(i) *Unrestricted random sampling.*—In a finite population containing N units, when 'n' units are selected at random, it is well known that the sample proportion 'p' is an unbiassed estimate of the population percentage P and its variance is given by

$$V(p) = \frac{N-n}{N-1}\frac{PQ}{n}. \qquad (1)$$

An estimate of the sample size required to attain a specified degree of precision is made as follows. The equation may be written as follows:

$$Cpp = \frac{V(p)}{P^2} = \frac{N-n}{N-1}\frac{Q}{nP} \qquad (2)$$

where C*pp* denotes the square of the coefficient of variation of p. If we specify the desired value of coefficient of variation, equation (2) may be solved for n.

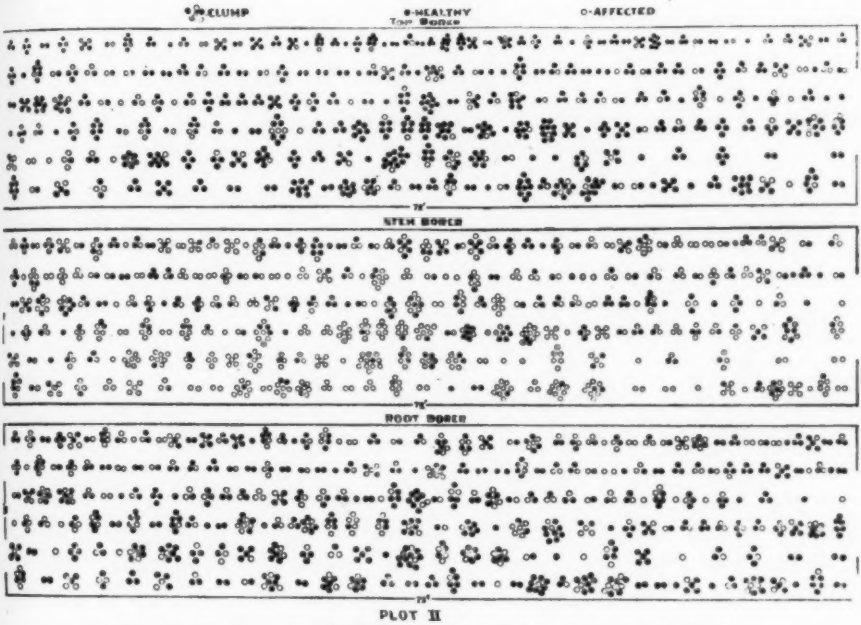

FIG. 2. Showing the distribution of borer infestation in the field.

(ii) *Stratified random sampling.*—Let N_i be the number of units in the ith stratum out of which n_i elements are selected at random ($\sum_i N_i = N$, $\sum_i n_i = n$) and let

$$P_i = \frac{A_i}{N_i} \quad \text{and} \quad p_i = \frac{a_i}{n_i}$$

be the proportions of elements in class C in the ith stratum and in the sample from that stratum respectively. For the proportion in the whole population the unbiassed estimate will be

$$p = \frac{\sum_i N_i p_i}{N}$$

and its variance will be given by

$$V(p) = \frac{1}{N^2} \sum_i \frac{N_i^2 (N_i - n_i)}{N_i - 1} \frac{P_i Q_i}{n_i}.$$

For proportional allocation in which $n_i/N_i = f$ it reduces to (assuming N_i to be large enough to permit the approximation $N_i - 1/N_i = 1$):

$$V(p) = \frac{1}{N^2} \sum_i N_i P_i Q_i \frac{1-f}{f} \tag{3}$$

and

$$Cpp = \frac{V(p)}{P^2} = \frac{1}{N^2 P^2} \sum_i N_i P_i Q_i \frac{1-f}{f} \tag{4}$$

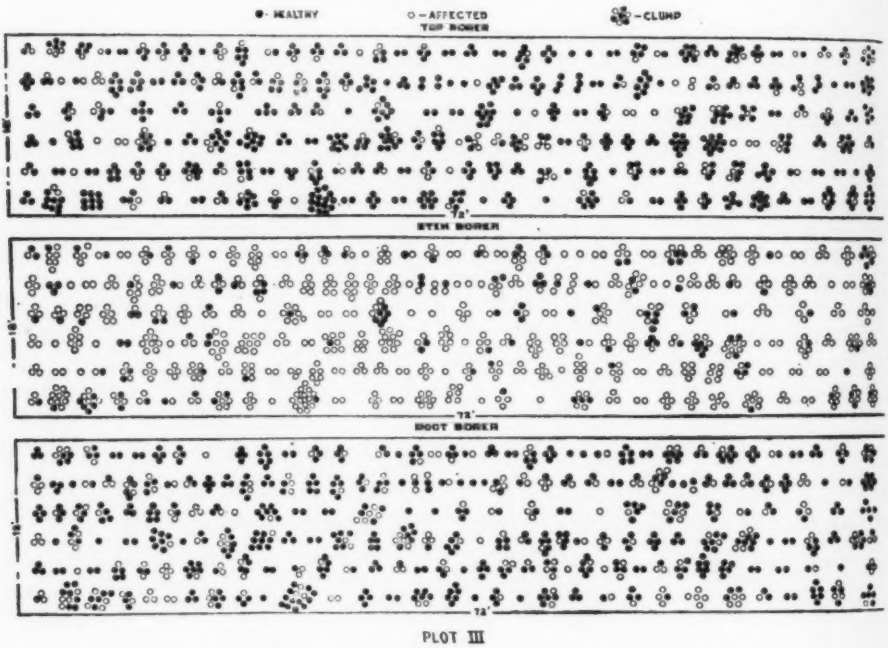

FIG. 3. Showing the distribution of borer infestation in the field.

An estimate of the sample size, required to attain a specified value of coefficient of variation, is made by solving for f in (4).

(iii) (*a*) *Cluster sampling* (*without subsampling*).—Let the ith cluster consist of M_i units ($\sum_i M_i = M$). Suppose that $p_i = a_i/M_i$ is the propotion of units in class 'C' in the ith cluster. Let n clusters be selected out of N with probability porportional to measures of size $Z_i = M_i/M$ and with replacement.

Then, $p = \sum_{i=1}^{n} 1/n\ p_i$ will be an unbiassed estimate of P, the population proportion and its variance will be given by

$$V(p) = \frac{1}{n} \sum_{i=1}^{N} \frac{M_i}{M} (p_i - P)^2 \tag{5}$$

and

$$Cpp = \frac{1}{nP^2} \sum_{i=1}^{N} \frac{M_i}{M} (p_i - P)^2. \tag{6}$$

An estimate of the sample size required to attain a specified value of coefficient of variation will be obtained by solving for n in (6).

(*b*) *Cluster sampling* (*with subsampling*).—Let n be the number of clusters sampled out of N clusters with replacement and probability proportional to measures of size $Z_i = M_i/M$. Also let a subsample of m elements be drawn by random sampling from each selected cluster. Suppose that $p_i = a_i/m$ is the proportion of C in the ith cluster if it is selected. Then $p = 1/n \sum_{i=1}^{n} p_i$ is an unbiassed estimate of P, the population propotion and its variance will be approximately given by

$$V(p) = \frac{1}{nM} \sum_{i=1}^{N} \left[M_i (P_i - P)^2 + \frac{M_i - m}{m} P_i Q_i \right] \tag{7}$$

where P_i is the population proportion in the ith cluster.

$$Cpp = \frac{1}{nMp^2} \sum_{i=1}^{N} \left[M_i (P_i - P)^2 + \frac{M_i - m}{M} P_i Q_i \right]. \tag{8}$$

(Cochran)

An estimate of the required sample size for a given value of C.V. and with m, the number of elements to be sampled from each cluster being fixed, can be obtained by solving for n in equation (8).

IV. Experimental Materials

Three plots, each of 1/33·6 acre in size (72′ × 18′), were marked out at random from three non-experimental cane fields. The first plot was under variety Co. 453 and the other two plots were given to B.O. 11. Each plot contained 6 rows 3′ apart. The plots were completely enumerated in respect

of number of clumps, total number of canes in each clump and number of canes affected in each clump by top, stem and root borers which was found by digging out the clumps and splitting the canes longitudinally. Subsequently stratum of 12′ row length was formed in each row, thus constituting 36 strata in the entire plot. Table I shows the total number of clumps and canes and the total number affected by each of the borers with their respective proportions of affected canes.

TABLE I

Showing number of clumps and canes, number affected by borers

Plots	I	II	III
No. of clumps ..	341	254	214
No. of canes ..	1067	892	808
No. affected—			
Top	205	170	134
Stem	248	577	640
Root	402	312	217
Proportion of total—			
Top	0·1928	0·1906	0·1658
Stem	0·2333	0·6469	0·7921
Root	0·3781	0·3496	0·2686

The percentages of canes affected by top borer are the least and almost equal in the first two plots. In the first plot incidence of root borer is the maximum, whereas in the others incidence of stem borer is the highest showing appreciably severe infestation. The distributions of borer infestation in the three plots have been shown in Figs. 1–3.

V. DISCUSSION OF RESULTS

Sample sizes for estimates with a level of accuracy, $^{\sigma}p/p = 0{\cdot}10$ have been calculated in respect of incidences of each of the three kinds of borers for unrestricted random sampling, stratified random sampling, cluster sampling in which the clusters are entirely enumerated and cluster sampling in two stages, sampling being done at the rate of two elements per cluster assuming that the clusters are sampled with probability proportional to size and replacement. The individual stools have been reckoned as clusters for this purpose. Beal[5] has obtained the smallest sample sizes for a given degree of accuracy under Neyman's Optimum Allocation, but as this design requires a prior knowledge of variances of each stratum which is not possible to acquire in this case through preliminary survey of the field, for it will neces-

sitate splitting of canes, sample sizes in respect of stratified sampling design have been found out under proportional allocation.

In Table II have been furnished the sample sizes obtained for the different sampling designs, the level of accuracy being $\sigma p/p = 0{\cdot}10$.

TABLE II

Showing percentage of total crop to be sampled for an estimate with 10% standard error

	Top			Stem			Root		
	Plot I	Plot II	Plot III	Plot I	Plot II	Plot III	Plot I	Plot II	Plot III
Unrestricted Random sampling	28·19	32·26	38·37	23·60	5·76	3·17	13·39	17·52	25·20
Stratified Random sampling	27·63	31·34	36·85	22·40	5·42	3·15	13·32	17·31	24·80
Cluster sampling (one stage)	40·18	58·27	76·11	35·77	7·48	7·96	15·83	20·47	41·15
Cluster sampling with sub-sampling	39·17	53·81	Impos-sible	35·05	6·95	6·93	16·68	21·75	37·13

The above results show that highest sample sizes are required under both kinds of cluster sampling being mostly one and half times and in two cases almost double the sizes obtained for the other two designs. There is practically no difference between enumeration of entire stools and sampling of portion of stools done in two stages. Both the methods have proved highly inefficient in comparison to random selection of individual stalks out of the entire material. This may be attributed to the inherent nature of the pests to damage in clusters making the intra-class correlation coefficients amongst elements within a cluster always highly positive. From a comparison of the sample sizes obtained under unrestricted random sampling and stratified random sampling designs it will be seen that reductions in the sample sizes due to stratification have been quite insignificant in all the plots and for all degrees of infestation, although the size of a stratum has been taken as small as 12′ row length indicating that no gain in efficiency can be secured through stratification. The results further indicate that with the increase in the degree of infestation, there is an appreciable reduction in the sample size, but these are independent of kind of insects.

VI. SUMMARY OF RESULTS

1. The efficiency of four sampling designs adopted for the purpose of estimating stalk basis incidence in a field of the three kinds of sugarcane borers has been examined.

2. For a given degree of accuracy sampling of entire clumps or of canes in two stages, clumps constituting the first sampling hierarchy has been found to be highly inefficient as compared to the unrestricted random sampling and stratified random sampling of canes with 12′ row length as the size of stratum, allocations of the total sample amongst the strata being made in proportion to the size.

3. As between random sampling of canes *vs.* stratified random sampling of canes there is practically no gain in efficiency due to stratification.

4. Sample sizes are independent of the kind of insects but are highly associated with the degree of infestation, being low for high infestation and high for low incidence.

VII. ACKNOWLEDGMENTS

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STUDIES ON INDIAN ICHNEUMONIDÆ (HYMENOPTERA)

Part I. Subfamily *Mesochorinæ*

Part II. Subfamily *Diplazoninæ*

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PART I

SUBFAMILY MESOCHORINAE

INTRODUCTION

IN a previous paper the importance of the parasitic family Ichrenomonidæ (Hymenoptera) has been stressed from the point of view of its applied

importance in the biological control of insect pests. No work has been done in India on the subfamily Mesochorinæ. Morley mentioned Mesochorides as tribe of Ophioninæ in *Fauna British India, Hymen.*, 3 (1913). This subfamily has now been created according to recent trends in the taxonomic work of this family. All the available references to the various species have been verified and the recent views of the specialists on the synonymy of this group has been adopted.

ZOOLOGICAL NOMENCLATURE

International rules on zoological nomenclature as supplemented by opinions have formed the basis for this systematic work. All the supergeneric names are based on the oldest included genus.

ACKNOWLEDGEMENT

Taxonomic work of this subfamily has been made possible under a generous grant sanctioned by the Indian Council of Agricultural Research. We are indebted to Prof. Townes, author of *A Catalogue and Reclassification of Nearctic Ichrenmonidæ* for his valuable suggestions and comments. We wish to thank Dr. B. P. Lal, Director, Indian Agricultural Research Institute, for the keen interest he has taken in this research work.

ARRANGEMENT

Superspecific classifications and 'Trivial' names have been adopted from Muesebeck *et al.* (1951) and described accordingly.

POSITION OF INDIAN MESOCHORINÆ

Davis (1897) treated *Mesochorus* Grav. in a tribe Mesochorini of the subfamily Ophioninæ. Ashmead (1900) treated *Mesochorus* Grav. besides other related genera in a tribe Mesochorini of the subfamily Ophioninæ. Dalla Torre (1901–02) put *Mesochorus* Grav. and related genera in a subfamily Mesochorinæ of Ophionoidæ section. Szepligeti (1911) treated *Mesochorus* Grav. and related genera in subfamily Mesochorinæ in group Mesochoroidæ (Ophionoidæ part). The Mesochorides have been treated by Morley (1913) as tribe of Ophioninæ. But the genus *Mesochorus* of Gravenhorst, Ratzeburg, and Halmgren has been raised by recent taxonomists, namely, Muesebeck, Krombein, Townes and others (1951) to the rank of a definite subfamily.

So far no work has been done on the Indian Mesochorinæ. Morley (1913) placed the genus *Tetragonalys* Morley in the tribe Paniscides of the subfamily Ophioninæ and the genera *Astrophromma* Forst, *Mesochorus* Grav. and

Edinsa Cam. in tribe Mesochorides of the subfamily Ophioninæ. Very recently the genera *Pseudochorus* Rao and *Mesochorella* Szepligeti have been put in the tribe Mesochorini. We are of opinion that a more natural system of classification would be to include them also along with four in the subfamily Mesochorinæ.

The members of this subfamily are readily recognised from the remainder of the Ichneumoni dæ by the peculiar shape of alar areolet which is oblique square (large Rhomboidal) that is to say that it has angle at the top, bottom and either side in place of two on radial nervures and two below as in Cryptinæ and by two stylid projections at the apex of the abdomen of male. Spiracles are at or behind the centre never before as in most Ophioninæ.

The Mesochorides are parasites on Lepidoptera and other Hymenpotera and have been bred from Chrysomelid and Curculionid, but are most often met with in an injurious role as hyperparasites of beneficial Braconids of the subfamily Microgasterinæ of caterpillar pests.

Systematically Arranged Catalogue of Indian Species

Subfamily **MESOCHORINÆ**

Mesochoroidæ Forster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **17**: 143, 170, (1868).

Mesochorinæ Dalla Torre, *Cat. Hymen.*, **3**: (1901–02). Szepligeti, *Genera Insect., fas.* 114: 42, (1911). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 748, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 405, (1951).

Cidaphus Forster

Cidaphus Forster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 149, (1868), (Type of genus: *Cidaphus thuringiacus* Brauns). Desig. by Viereck, 1914. Townes, *Mem. Amer. ent. Soc.*, **11** (2): 748, (1945). Muesebeck, Krombein and others, *U.S. Dep. Agric. Monograph*, **2**: 405, (1951).

Plesiophthalmus Forster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 170, (1868). Preoccupied by Motschoulsky, (1858). Townes, *Mem. Amer.*

ent. Soc., **11** (2): 748, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 405, (1951).

Mater Schulz, *Zool. Ann.*, **4**: 44, (1911). (New name for *Plesiophthalmus* Forster.) Townes, *Mem. Amer. ent. Soc.*, **11** (2): 748, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 405, (1951).

Tetragonalys Morley, *Rev. Ichn. Brit. Mus.*, **2**: 132, (1913); *Fauna Brit. India, Hymen.*, **3**: 359, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (2): 748, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 405, (1951).

[1] Range. Himalayas.

Plesiophthalmidea Viereck, *Bull. U.S. Nat. Mus.*, **83**: 119, (1914). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 748, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 405, (1951).

Ophthalmochorus Rohman, *Archivfor Zoologi*, **17 A** (4): 29, (1925). N. name for *Plesiophthalmus*. Townes, *Mem. Amer. ent. Soc.*, **11** (2): 748, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 405, (1951).

barbarica (Morley), n. comb.

Tetragonalys barbarica Morley, *Rev. Ichn. Brit. Mus.*, **2**: 132, (1913).[1]

[1] Tibet: Gyangtse.

Astiphromma Forster

Astiphromma Forster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 170, (1868), (Type of genus: *Mesochorus scutellatus* Gravenhorst). Dalla Torre, *Cat. Hymen.*, **3**: 60, (1901–02). Szepligeti, *Genera Insect., fas.* **114**: 43, (1911). Morley, *Ichn. Brit.*, **5**: 306, (1913); *Fauna Brit. India, Hymen.*, **3**: 516 (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (2): 749, (1945). Muesebeck,

[1] United States, Europe, Ceylon,

Krombein, Townes and others, *U.S Dep. Agric. Monograph*, **2**: 405, (1951).

Astiphrommus Thomson, *Soc. Ent. France, Ann.*, **5** (6): 327, (1886).

petiolata Morley

Astiphromma petiolata Morley, *Fauna Brit. India, Hymen.*, **3**: 516, (1913). ♀[1].

[1] Ceylon: Peradeniya.

Mesochorus Gravenhorst

Mesochorus Gravenhorst, *Echn. Eur.*, **2**: 960, (1829), (Type of genus: *Mesochorus splendidulus* Grav.) Dalla Torre, *Cat. Hymen.*, **3**: 51, (1901–02). Szepligeti, *Genera Inesct., fas.* **114**: 44, (1911). Morley, *Ichn. Brit.*, **5**: 313, (1906); *Fauna Brit. India, Hymen.*, **3**: 517, (1913).[1] Dutt, *Mem. Dep. Agric. India, Ent.*, **8** (2): 22, (1923). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 750, (1945). Muesebeck, Krombein, Townes, and others, *U.S. Dep. Agric. Monograph*, **2**: 406, (1951).

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claristigmaticus Morley

Mesochorus claristigmaticus Morley, *Fauna Brit. India, Hymen.*, **3**: 519, (1913). ♀.[1]

[1] Sikkim: Darjeeling.

confusus Holmgren

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[1] Assam: Shillong.

facialis Bridgman

Mesochorus facialis Bridgman, *Trans. Ent. Soc. Lond.*, 431, (1884). Thomson, *Soc. Ent. France Ann.*, 343, (1886). ♂, ♀. Dalla Torre,

Cat., Hymen., **3**: 54, (1901-02). Szepligeti, *Genera Insect., fas.* **114**: 45, (1911). Morley, *Ichn. Brit.*, **5**: 334, (1913); *Fauna Brit. India, Hymen.*, **3**: 518, (1913). ♂, ♀.[1] Dutt, *Mem. Dep. Agric. India, Ent.*, **8** (2): 22, (1923).[2]

[1] Bengal: Pusa, Chopra; Assam: Sadiya, Europe.
[2] Bengal: Pusa, Chopra.

fragilis Morley

Mesochorus fragilis Morley, *Fauna Brit. India, Hymen.*, **3**: 519, (1913). ♂.[1]

[1] Tenasserim.

hapaliæ Rao

Mesochorus hapaliæ Rao, *Indian For. Rec.*, New series, **8** (8): 200, (1953). ♀.[1]

[1] Wynaad (Madras).

indica Cushman

Mesochorus indica Cushman, *Indian For. Rec.* Delhi, **20** (12): 8, (1934). ♂, ♀.[1]

[1] Rohatgoon, Hoshangabad, Central Provinces, India.

panti Rao

Mesochorus panti Rao, *Indian For. Rec.*, New series, **8** (8): 198, (1951). ♀.[1]

[1] Wynaad (Madras).

Edrisa Cameron

Edrisa Cameron, *Tijds. Ent.*, 111, (1907), (Type of genus: *E. pilicornis* Cam.). Morley, *Fauna Brit. India, Hymen.*, **3**: 520, (1913).[1]

[1] Range. Sikkim.

pilicornus Cameron

Ednisa pilicornus Cameron, *Tijds. Ent.*, 112, (1907), ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 521, (1913). ♀.[1]

[1] Sikkim.

Pseudochorus Rao

Pseudochorus Rao, *Indian For. Rec.* New series, **8** (8): 195, (1953), (Type of genus: *Pseudochorus kuriani* Rao).

kuriani Rao

Pseudochorus kuriani Rao, *Indian For. Rec.*, New series, **8** (8): 195, (1953). ♂, ♀.[1] [1] Agra (U.P.).

Mesochorella Szepligeti

Mesochorella Szepligeti, *Genera Insect., fas.* **114**: 48, (1911), (Type of genus: *M. nigriceps* Brischke).

indica Rao

Mesochorella indica Rao, *Indian For. Rec.*, New series, **8** (8): 201, (1953). ♀.[1] [1] Dehra Dun (U.P.).

PART II

Subfamily **DIPLAZONINÆ**

The Bassides have been given as tribe of Tryphoninæ by Morley (1913). It has now been raised to the subfamily Diplazoninæ in accordance with the recent trends in the taxonomic work of this family. No work has been done in India on the subfamily Diplazoninæ. They have common host relationship namely Sphryphidæ and not Tenthredinidæ which is mostly attacked by the members of the subfamily Tryphoninæ. They oviposit into the egg or young larva or the host and emerge from puparium. The adults have the head broader than deep, clypeus separated, mandibles, which have the superior tooth divided or notched at the tip, so that in appearance there are 3 teeth to each mandible, antennæ filiform composed of from 20 to 25 joints, normal scutellum and metathorax in part Carinated. The peculiar conformation of the strongly deplanate and sessile basal segment is also distinctive.

Systematically Arranged Catalogue of Indian Species

Subfamily **DIPLAZONINÆ**

Diplazoninæ Townes, *Mem. Amer. ent. Soc.*, **11** (2): 553, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 349, (1951).

Diplazon Nees

Bassus of authors and not of the type of genus. *Diplazon* Nees, *Acta Acad. Nat. Nov.*, **9**: 202,

(1818), (Type of genus: *Ichneumon lætatorius* Fabricius). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 553, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 348, (1951).

clotho (Morley), n. Comb.

Bassus clotho Morley, *Fauna Brit. India, Hymen.*, **3**: 281, (1913). ♂, ♀.[1]

[1] Kashmir, 6000-7000 ft.; Bengal: Pusa; Central Provinces: Nagpur, Bombay: Surat and Poona.

lætatorius (Fabricius) n. Comb

Ichneumon lætatorius Fabricius, *spec. Ins.*, **1**: 424, (1781); *Mant. Ins.*, **1**: 262, (1787); *Piez.*, 63, (1804). Panzer, *Faun. Germ.*, **19**: 19. Villers, *C. Linnæi Entom.*, **3**: 150, n. 46, (1789). Gmelin, *Linne*: *Syst. Nat. Ed.*, 13ª, **1** (5): 2682, n. 99, (1790). Rossi, *Mant. Insect.*, 116, n. 254, (1792). Olivier, *Encycl. Method. Insect.*, **7**: 220, n. 83, (1792). Cederhjelm, *Faun. Ingric Prodr.*, 158, n. 487, (1798). Schrank, *Fauna Boica II*, **2**: 287, n. 2097, (1802). Schnokenstein, *Verz. d. Halbkofer*, etc., 25, n. 68, (1802). Latreille, *Hist. Nat. Insect.*, **13**: 185, n. 21, (1805). Lamarck, *Hist. Nat. aim.*, 8 vert., **4**: 116, (1807). ♀. Thanberg, *Bull. Acad. Sc. st. Peterburg*, **7**: 277, (1822); *Mem. Acad. Sc. st. Peterburg*, **9**: 353, (1824). Trentopol, *Isis*, 298, n. 64, (1826). ♀. V. D. Hoven, *Bijdr. natuurk wet.*, **1**: 439, n. 12, (1826). Morley, *Fauna Brit. India, Hymen.*, **3**: 276, (1913). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 553, (1945).

Ichneumon dichrous Schrank, *Ent. Insect. Austr.*, 352, n. 710, (1781). ♂. Gmelin, *Linne. Syst. Nat. Ed.* 13ª, **1** (5): 2683, n. 110, (1790).

Bassus lætatorius Panzer, *Krit. Revis.*, **2** : 74, (1806). Gravenhorst, *Ichn. Eur.*, **3**: 353, (1829). Zetterstedt, *Ins. Lapp.*, 377. Ratzeburg, *Ichn. d. Forst.*, **2**: 109. Blanchand, *Hist. nat. Insect.*, **3**: 321, 461, (1840). Westwood, *Introd. mad. classif. Insect. II* Synops., 59, (1840). Holmgren, *Svenska. Vet. Akad. Handl.*, pl. 9, fig. 24, 353, (1855). Vallenhoven, *Pinac.*, pl. 1, fig. 1. Thomson, *Opusc. ent.*, **14**: 1470 (|), Fyles, *Ann. Rpt. Ent. Soc. Ent.*, **25**: 54, (1894). Davis, *Trans. Amer. Ent. Soc.*, **22**: 19, (1895). Bridwell, *Trans. Kans. Acad. Sci.*, **16**: 204, (1899). Ashmead, *Ann. Rpt. N.Z. Bd. Agr.* 27 Suppl., 579, (1900). Johnson, *Canad. Ent.*, **32**: 59, (1900). (|) Viereck, *Trans. Kans. Acad. Sci.*, **17**: 292, (1905); *Ann. Rpt. N.J. Mus.*, for 1909, 1910, p. 622. Nason, *Ent. News*, **17**: 169, (1905). Morley, *Trans. Ent. Soc.*, 425, (1905). ♂, ♀; *Fauna Brit. India, Hymen.*, **3**: 276, (1913);[1] *Rev. Ichn. Brit. Mus.*, **3**: 125, (1914). (|) Tucker, *Trans. Kans. Acad. Sci.*, **22**: 291, (1909). Roman, *Ent. Tidjskr.*, **31**: 149, (1910).

[1] Baluchistan: Quetta; Kashmir; Punjab: Gojra, Ferozepur; Rajputana. Cosmopolitan.

Cryptus lætatorius Lamarck, *Hist. Nat. aim. S. vert.*, **4**: 116, (1807). ♀.

Anomalon lætatorius Jurine, *Nouv. Meth.*, 116, (1807). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 276, (1913). (|) Washburn, *Ann. Rpt. state Ent. Minn.* **17**: 172, (1918). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 554, (1945).

Anomalon attractus Say, *Boston Jour. Nat., Hist.*, **1**: 241, (1836). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 553, (1945).

Bassus albovarius Wolloston, *Ann. Nat. Hist.*, **1**: 23, (1858). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 277, (1913).

Bassus sycophanta Cression, *Trans. Amer. Ent. Soc.*, **2**: 112, (1868). ♂, ♀; *Trans. Amer. Ent. Soc.*, **4**:

167, (1872); Walsh, *Trans. Acad. Sci. St. Louis*, **3**: 86, (1873). ♀. Provancher, *Nat. Canad.*, **6**: 31, 56, (1874); *Nat. Canad.*, **11**: 276, (1879); *Nat. Canad.*, **16**: 24, (1887). Ashmead, *Bull. Colo. Biol. Ass.*, **1**: 44, (1890). Cook, *Ann. Rpt. Mich. Agr. Exp. Sta.*, **4**: 132, (1891). Riley and Howard, *Insect Life*, 3: 450, (1891). Bidwell, *Trans. Kans. Acad. Sci.*, **16**: 204, (1899). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 554, (1945).

Bassus tripicticrus Walsh, *Trans. Acad. Sci. St. Louis*, **3**: 85, (1873). ♂, ♀. Morley, *Fauna Brit. India, Hymen.*, **13**: 277, (1913). Fyles, *Ann. Rpt. Ent. Soc. Ont.*, **46**: 57, (1916). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 554, (1945).

Scolobates varipes Smith, *Trans. Ent. Soc., Lond.*, 3, (1878). Morley, *Fauna Brit. India, Hymen.*, **3**: 277, (1913).

Bassus ventulus Saussure, *Grandidier, Hist. Madagascor Hym.*, pl. 15, fig. 23, ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 277, (1913).

Diplazon lætatorius Kelley, *Jour. Econ. Ent.*, **7**: 294–97, (1914). McGregor and McDonough, *Bull. U.S. Dep. Agric.*, **416**: 53, (1917). Viereck, *Bull. Cann. Geol. Nat. Hist. Surz.*, **22**: 303, (1917). ♀. Hauser, Guyton and Lowry, *Bull. Ohio Agr. Exp. Sta.*, **317**: 80, (1917). (l) Davidson, *Canad. Ent.*, **51**: 236, (1919). Cushman and Gahan, *Proc. Ent. Soc. Wash.*, **23**: 154, (1921). Campbell and Davidson, *Bull. So. Calif. Acad. Sci.*, **23**: 9, (1924). Kamal, *Jour. Econ. Ent.*, **19**: 724, 727, (1926). Bradley, *Bull. Lloyd. libr.*, **27**: 170, (1926). Cushman, *Proc. Ent. Soc. Wash.*, **28**: 44, (1926); *Mem. Cornell Univ. Agr. Exp. Sta.*, **101**: 249, (1928). Johnson, *Biol. Surv. Mt. Desert Region*, **1**: 136, (1927); *Pub. Nantucket Maria Mitchell Ass.*,

2 (3): 103, (1930). Criddle, *Ann. Rpt. Ent. Soc. Ont.*, **58**: 102, (1928). Poos, *Proc. Ent. Soc., Wash.*, **30**: 150, (1928). Fluke, *Res. Bull. Wis. Agr. Exp. Sta.*, **193**: 23, 42, (1929). Schopp, *Jour. Kans. Ent. Soc.*, **4**: 35, (1931). Cole, *Jour. Econ. Ent.*, **26**: 862, (1933). Heiss, *Ill. Bio. Monogr.*, **36** (1): 19, 99, 100, (1938). Brimley, *Insect. of N.C.*, 413, (1938). Telford, *Bull. Minn. Agr. Exp. Sta.*, **140**: 10, (1939). Knowlton and Hormston, *Proc. Ut. Acad. Sci. Arts Letters*, **16**: 61, (1939). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 553, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph.*, **2**: 348, (1951).

Diplazon sycophanta Viereck, *Bull. Conn. Geol. Nat. Hist. Surv.*, **22**: 303, (1917). ♂, ♀. Townes, *Mem. Amer. ent., Soc.*, **11** (2): 554, (1945).

multicolor (Gravenhorst), n. Comb.

Bassus multicolor Gravenhorst, *Ichn. Eur.*, **3**: 352, (1829). ♀. Vollenhoven, *Pinac.*, pl. 1, fig. 4. ♂. Holmgren, *Svenska. Vet. Akad. Handl.*, 335, (1855). Brischke, *Schrift. Naturf. Ges. Danzig., N.F.*, **4** (3): 111, (1878). ♂. Jacob and Tosquinet, *Ann. Soc. Entom. Belgique*, **14**: 119, (1890). Dalla Torre, *Cat. Hymen.*, **3**: 248, (1901–02). Morley, *Trans. Ent. Soc. Lond.*, 425, (1905). ♂, ♀. *Fauna Brit. India, Hymen.*, **3**: 279, (1913).[1]

[1] Bengal: Patna and Pusa; Bombay. Europe.

Bassus deletus Thomson, *Opusc. ent.*, **14**: 1471, (1890). ♂, ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 279, (1913).

orientalis (Cameron), n. Comb.

Bassus orientalis Cameron, *Spol. Zeyl.*, 731, (1905). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 280, (1913). ♂.[1]

[1] Assam: Shillong; Bengal: Bettia and

tricinctus (Gravenhorst), n. Comb. — Katihor; Ceylon: Peradeniya.

Bassus albosignatus var. 4. Gravenhorst, *Ichn. Eur.*, **3**: 345, (1829). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 278, (1913).

Bassus tricinctus Gravenhorst, *Ichn. Eur.*, **3**: 351, (1829). ♂. Dalla Torre, *Cat. Hymen.*, **3**: 251, (1901–02). Morley, *Trans. Ent. Soc. Lond.*, 425, (1905). ♂. ♀; *Fauna Brit. India, Hymen.*, **3**: 278, (1913).[1] *cf.* Brischke, *Schr. Nat. Ges. Danz.*, 111, (1878). — [1] Sikkim: Darjeeling; Bengal: Kotihar; Rajputana: Mt. Abu. Palæarctic Region.

Bassus flavolineatus Zetterstedt (nec Grav.), *Ins. Lapp.*, **1**: 378, excl. ♀ et var. Morley, *Fauna Brit. India, Hymen*, **3**: 278, (1913).

Bassus nemoralis Holmgren, *Svenska. Vet. Akad. Handl.*, 354, (1855); Vollenhoven, *Pinac.*, pl. 1, fig. 3. Thomson, *Opusc. ent.*, **14**: 1469, ♂, ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 278, (1913).

varicoxa (Thomson), n. Comb.

Bassus albosignatus var. 1. Gravenhorst, *Ichn. Eur.*, **3**: 344, (1829). ♀. (?); Var. 3, Holmgren, *Svenska. Vet. Akad. Handl.*, 354, (1855). Morley, *Fauna Brit. India, Hymen.*, **3**: 278, (1913).

Bassus varicoxa Thomson, *Opusc. ent.*, **14**: 1640, (1890). Morley, *Fauna Brit. India, Hymen.*, **3**: 278, (1913).

Syrphoctonus Forster

Syrphoctonus Forster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 162, (1868), (Type of genus: *Bassus biguttatus* Gravenhorst). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 561, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 350, (1951).

Homotropus Forster, *Verh. Naturh. Ver. Preuss. Rheinl.*, 162, (1868) (Part). Morley, *Fauna Brit. India, Hymen.*, **3**: 282, (1913).

Homoporus Thomson, *Opusc. ent.*, **16**: 1488, (1890). Preoccupied by Thomson, 1878. Townes, *Mem. Amer. ent. Soc.*, **11** (2): 561, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 350, (1951).

Homocidus Horley, *Ichn. Brit.*, **4**: 87, (1911); *Fauna Brit. India, Hymen.*, **3**: 282, (1913).[1] New name for *Homoporus* Thomson. Townes, *Mem. Amer. ent. Soc.*, **11** (2): 561, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 350 (1951).

[1] Range. Palæarctic and Nearctic Regions.

cinctus (Gravenhorst), n. Comb.

Bassus cinctus Gravenhorst, *Ichn. Eur.*, **3**: 327, (1829). Holmgren, *Svenska. Vet. Akad. Handl.*, 356, (1855). Vollenhoven, *Pinac.*, pl. 1, fig. 6, ♂; *Tijds. Ent.*, 162, (1878). ♀. Morley, *Trans. Ent. Soc. Lond.*, 426, (1905). ♂. ♀; *Fauna Brit. India, Hymen.*, **3**: 283, (1913).[1]

[1] Sikkim: Darjeeling. Europe.

Bassus lateralis Gravenhorst, *Ichn. Eur.*, **3**: 342, (1829). ♂. Holmgren, *Svenska. Vet. Akad. Handl.*, 355, (1855) Vollenhoven, *Pinac.*, pl. 1, fig. 5, ♂, ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 283, (1913).

Bassus albicinctus Desvignes, *Trans. Ent. Soc. Lond.*, 218, (1862). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 283, (1913).

Bassus hyperboreus Marshall, *Ent. Month. Mag.*, **13**: 241, (1877). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 283, (1913).

Homoporus lateralis Thomson, *Opusc. ent.*, **14**: 1492, (1890). ♂, ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 283, (1913).

Homoporus cinctus Morley, *Trans. Ent. Soc. Lond.*, 426, (1905), ♂, ♀. *Fauna Brit. India, Hymen.*, **3**: 283, (1913).

dimidiatus (Schrank), n. Comb.

Ichneumon dimidiatus Schrank, *Fauna Boica*, **2**: 293, Gravenhorst, *Ichn. Eur.*, **3**: 950, (1829). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 296, (1913).

Bassus pictus Gravenhorst, *Ichn. Eur.*, **3**: 336, ♂, ♀. Holmgren, *Svenska. Vet. Akad. Handl.*, 361, (1855). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 286, (1913).

Bassus planus Desvignes, *Trans. Ent. Soc. Lond.*, 220, (1862). ♂, ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 286, (1913).

Homoporus pictus Thomson, *Opusc. ent.*, **14**: 1511, (1890): Morley, *Fauna Brit. India, Hymen.*, **3**: 286, (1913).

Homoporus dimidiatus Morley, *Trans. Ent. Soc. Lond.*, 428, (1905). ♂, ♀.; *Fauna Brit. India, Hymen.*, **3**: 286, (1913).

Homocidus dimidiatus Morley, *Fauna Brit. India, Hymen.*, **3**: 286, (1913).[1]

[1] Sikkim: Darjeeling. Europe.

ornatus (Gravenhorst), n. Comb.

Bassus deplanatus Grevenhorst, *Ichn. Eur.*, **3**: 340, (1829). (excl. ♂). Morley, *Fauna Brit. India, Hymen.*, **3**: 285, (1913).

Bassus ornatus Gravenhorst, *Ichn. Eur.*, **3**: 341, (1829). Brischke, *Schr. Nat. Ges. Danz.*, 113, (1878). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 285, (1913).

Bassus frenator Desvignes, *Trans. Ent. Soc., Lond.*, 218, (1862). ♂. Morley, *Fauna Brit, India, Hymen.*, **3**: 285, (1913).

Homoporus ornatus Thomson, *Opusc. ent.*, **14**: 1505, (1890). Morley, *Trans. Ent. Soc., Lond.*, 427, (1905). ♂, ♀. *Fauna Brit. India, Hymen.*, **3**: 285, (1913).

Var. *Bassus deplanatus* Holmgren, *Svenska. Vet. Akad. Handl.*, 362, (1855). Morley, *Fauna Brit. India, Hymen.*, **3**: 285, (1913).

Homocidus ornatus Morley, *Fauna Brit. India, Hymen.*, **3**: 285, (1913).[1]

[1] Punjab: Kangra Valley.

Tarsatorius (Panzer), n. Comb.

Bassus tarsatorius Panzer, *Faun. Germ.*, **9**: 102, (1805). Gravenhorst, *Ichn. Eur.*, **3**: 293, (1829). Morley, *Fauna Brit. India, Hymen.*, **3**: 283, (1913).

Bassus exultous Gravenhorst, *Ichn. Eur.*, **3**: 328, (1829). ♂. Ratzeburg, *Ichn. d. Forst.*, **1**: 122. Holmgren, *Svenksa. Vet. Akad. Handl.*, 359, (1855). ♂. ♀. excl. var. l. Morley, *Fauna Brit. India, Hymen.*, **3**: 283, (1913).

Bassus insignis Gravenhorst, *Ichn. Eur.*, **3**: 349, (1913). Holmgren, *Svenska. Vet. Akad. Handl.*, 360, (1855). Brischke, *Schr. Nat. Ges. Danz.*, 112, (1878). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 284, (1913).

Bassus pulchellus Desvignes (nec. Holmg.), *Trans. Ent. Soc. Lond.*, 221, (1862). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 284, (1913).

Bassus desvignesii Marshall, *Cat. Brit. Hymen.*, 82, (1872). Morley, *Fauna Brit. India, Hymen.*, **3**: 284, (1913).

Homoporus tarsatorius Thomson, *Opusc. ent.*, **14**: 1503, (1890). Morley, *Trans. Ent. Soc. Lond.*, 427, (1905). ♂, ♀.; *Fauna Brit. India, Hymen.*, **3**: 284, (1913).

Bassus indicus Cameron, *Journ. Bombay Nat. Hist. Soc.*, 728, (1909). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 284, (1913).

Var. *Bassus flavus* Desvignes, *Trans. Ent. Soc., Lond.*, 219, (1862). ♂.

Homocidus tarsatorius Morley, *Fauna Brit. India, Hymen.*, **3**: 283, (1913).[1]

[1] Punjab: Simla. Europe.

Promethes Forster

Promethes Forster, *Verh. Naturh. Ver. Preuss.*, **25**: 162, (1868), (Type of genus: *Bassus sulcator* Gravenhorst). Dalla Torre, *Cat. Hymen.*, **3**: 242, (1901–02). Morley, *Fauna Brit. India, Hymen.*, **3**: 287, (1913). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 559, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 349, (1951).

Liopsis Forster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 162, (1868). Townes, *Mem. Amer. ent. Soc.*, **11** (2): 559, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph.*, **2**: 349, (1951).

Promethus Thomson, *Opusc. ent.*, **14**: 1465, 1475, (1890). Morley, *Fauna Brit. India, Hymen.*, **3**: 287, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (2): 559, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 349, (1951).

[1] Range. Palæarctic and Oriental Regions.

Sussaba Cameron, *Jour. Bombay Nat. Hist. Soc.*, **19**: 728, (1909). Morley, *Fauna Brit. India, Hymen.*, **3**: 287, (1913).

poonænsis Rao

Promethus poonænsis Rao, *Indian For. Rec., New Series*, **8** (8): 186, (1913).[1]

[1] Bangalore.

pulchellus Holmgren

Bassus sulcater Var. 1 ♂, Vars. 3 et. 4. ♂, ♀. Gravenhorst, *Ichn. Eur.*, **3**: 321, (1829),

Morley, *Fauna Brit. India, Hymen.*, **3**: 288, (1913).

Bassus festivus Vars. 2 et 3. Gravenhorst, *Ichn. Eur.*, **3**: 316, (1829). ♂, ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 288, (1913).

Bassus pulchellus Holmgren (nec. Desv.), *Svenska. Vet. Akad. Handl.*, 366, (1955). ♂, ♀. Brischke, *Schr. Nat. Ges. Danz.*, 113, (1878). Morley, *Fauna Brit. India, Hymen.*, **3**: 288, (1913).

Promethus pulchellus Thomson, *Opusc. ent.*, **14**: 1483, (1890). Morley, *Trans. Ent. Soc., Lond.*, 429, (1905). ♂, ♀; *Fauna Brit. India, Hymen.*, **3**: 288, (1913).[1]

Sussaba bicarinata Cameron, *Jour. Bombay Nat. Hist. Soc.*, 728, (1909). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 288, (1913).

[1] Punjab: Simla. Europe.

THE ANTENATAL DEVELOPMENT OF THE HUMAN PELVIS IN RELATION TO SEX DIMORPHISM

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Embryological investigations on the growth of the pelvis have been done by several observers. Bardeen (1905) traced the growth of the lower limb buds in the human embryos. In the 9·1 mm. C.R.L. stage, he described the condensation of the limb bud at the centre determined by the disposition of the branches of the lumbosacral plexus around it. He observed that a pelvic fundament was formed at the 12·5 mm. stage with the dorsal extension of the iliac blastema, the formation of the auricular plate of the sacrum, the growth of the ischial and pubic processes and the embryonic formation of the Poupart's ligament. At 14 mm. C.R.L. stage he stated that the three cartilages were distinct and that the pubic cartilages were completely joined in front in the third month.

Edmund Falk (1908) studied the growth of the fœtal pelvis from the eighth week period onward. He observed that the growth of the pelvis is pronounced considerably. The anterior superior iliac spine is formed in the fourth month and the intervertebral disc between the 5th lumbar and the 1st sacral vertebræ pushed more ventrally into the inlet of the pelvis; the diameters at the outlet decrease relatively. He gave several points of difference in the fœtal pelvis of the two sexes such as the shape of the obturator foramen, the increased sub-pubic angle, the increased inter-ischio-spinal diameter, the increased transverse diameter at the inlet and the increased distance between the centres of the acetabulæ in the female pelvis.

Adair and Scammon (1921) studied the fœtal bones of the pelvis and referred to the earlier occurrences of the epiphyses in the female.

Parson (1903), Patterson (1929) and Pryor and Kentucky (1928) have also observed earlier occurrences as well as earlier fusion of the epiphyses.

E. L. Reynolds (1945) made a rœntgenological study of the bony pelvic girdle in the newborn child and observed the following points:

1. That a large bi-ischial diameter is associated with a heavier and longer infant.

2. A significant positive relationship is found between the inlet transverse diameters of the pelves in the newborn infant and the mother.

The present enquiry was undertaken to study the development of the pelvis in its chronological stages with particular reference to the various features which form the sex patterns in the human pelvis during the antenatal period.

Material and Method

The growth pattern of the pelvis was studied in the different C.R.L. stages by microscopic examination of the serial sections belonging to the respective embryos. These sections stained with Hæmatoxylin and Eosin belonged to the Department of Anatomy, Glasgow University. The 100 mm. C.R.L. stage sections also stained by Hæmatoxylin and Eosin were kindly loaned for study by the late Professor Walmsley of Dublin.

Micrometric measurements were made on the embryos of 42 mm. and 100 mm. C.R.L. stages. Three wax model reconstructions of embryos in the 23 mm., 42 mm. and 100 mm. C.R.L. stages were prepared. Details of the architecture of the pelves were studied in these specimens.

The wax plates were 1 mm. thick. The magnification of the slides used was 50. The thickness of the sections was 10 μ. Hence alternate sections were taken to cut wax sections for the wax reconstruction model.

For the 100 mm. specimen the magnification used was 20 and the thickness of the section was 10 μ and so one out of every five consecutive sections was used to make the wax reconstruction model.

Descriptions of the Serial Sections of the Embryos

4·5 *mm. C.R.L. Stage*

The early growth of the lower limb bud is seen. It commences from the ventrolateral aspect of the somatopleure. It is situated adjacent to the intermediate cell mass which separates it from six consecutive segments of the paraxial somites. The serial number of these paraxial somites could not be counted because No. 5 slide was missing out of a total of 14 slides comprising the complete sections of this embryo.

16·1 *mm. C.R.L. Stage*

All the ribs have formed. The vertebræ in the sacrococcygeal region show the commencement of the cartilaginous stage.

The cartilaginous portion of the pelvis capping the head of the cartilaginous dumb-bell-shaped femur is seen (Fig. 1). The future ischial portion of the pelvis is seen adjoining the posterior part of the femoral head and it is growing downward.

The femoral nerve is seen coursing over the anterior aspect of the limb with the pre-muscle mass of the psoas which lies medial to it.

The course of the obturator nerve is observed entering the limb on its medial aspect. The portion of the pelvis overlying the femoral head is in a pre-cartilaginous state; it is seen to proceed backwards in the form of a pointed process (Fig. 1). This process is directed towards the 25th vertebra. Flanking the lateral margin of the sacrum at the level of the 3rd piece is seen the articular surface of the posterior medial margin of the ilium (Fig. 2). The lateral part of the sacrum is composed of mesenchyme and the articulating piece of the ilium is pre-cartilaginous.

The portions of the pelvis capping the head of the femur and the elongated process of the ilium are found to be in the pre-cartilaginous stage. The portions of the pelvis above and below these levels are found as a mesenchymatous mass. The region of the symphysis pubis is seen as dense mesoderm. This section of mesoderm pertains to the continuous sheet of mesoderm passing downwards on either side of the cloacal membrane from the caudal part of the body stalk which is primary mesoderm. The mesoderm passing upwards from the region of the termination of the primitive streak (Wyburn, 1937) is secondary mesoderm.

The sciatic nerve with its accompanying artery passes down along the posterior aspect of the limb.

The gonads.—The gonads are immature. There is a predominating growth of the medullary pattern.

The urogenital ducts are seen. The Wolffian ducts join the urogenital sinus.

The Mullerian ducts are seen only until the level of the 3rd lumbar vertebra.

23 *mm. C.R.L. Stage*

This is an abnormal embryo. It presents the vertebral column deviated to the right of the middle line. The vertebral bodies have

undergone torsion. In the coccygeal region there is a slight twist to the left (Fig. 3).

Posteriorly the ilia articulate with the first two sacral pieces. The pubic symphysis is widely parted. There is a double rotation of the pelvic girdle as brought out in the wax reconstruction model where the right half of the pelvis is tilted upwards and backwards and the left half is pulled downwards and rotated forwards.

The gonads.—These present the appearance of the testes with the epithelial cords and the thick tunica albuginea.

The urogenital ducts.—The urogenital folds meet in the middle line without any peritoneal excavation.

The Wolffian ducts open into the bladder. The ureters join the bladder more laterally.

The pelvic floor is not formed. The mesoderm is not differentiated. The blood vessels of this zone are seen very clearly.

42 mm. C.R.L. Stage

The full cartilaginous pattern of the pelvis is exhibited in the several slides pertaining to this embryo.

The 12 ribs are present on both sides. The vertebræ are distinguished in the several segments.

The sacral foramina are seen clearly and their measurements are noted (Table I B).

The sacro-iliac joint.—Three pieces of the sacrum articulate with the ilium.

The fibrous filaments of the future anterior sacro-iliac interrosseus and external parts of the posterior sacro-iliac ligaments are well seen.

The pattern of the symphysis pubis is clearly formed (Fig. 4) and the brim of the true pelvis is well defined. The ischium has grown downwards below and behind the acetabulum.

The urogenital ducts.—The Wolffian ducts are more predominant than the Mullerian ducts. The Mullerian ducts fuse in the middle line in the zone of the genital cord.

The uterus masculinus is clearly observed in this embryo above the bladder. The entry of the terminal portions of the Wolffian ducts into the bladder is also observed (Fig. 5).

TABLE I A

Micrometric measurements of the fœtal pelvis

	100 mm.	42 mm.
1. Maximum distance between highest portions of ilia ..	17·5	4·6
2. Width of 1st piece of sacrum ..	9·3	3·0
3. Width maximum at inlet (at level of upper border of 1st sacral vertebra) ..	17·5	4·2
4. Distance between lateral walls of pelvic cavity at the upper level of the acetabulum ..	6·5	2·5
5. Distance between lateral walls of pelvic cavity at the lower level of the acetabulum ..	5·7	2·0
6. Distance between lowest sections of ischial cartilages between their inner margins ..	5·5	2·0
7. Width of last piece of sacrum ..	3·0	2·2
8. Distance between posterior superior iliac spines ..	8·75	6·7

TABLE I B

Micrometric measurements of the fœtal pelvis

Vertical measurements, details of items	42 mm.	100 mm.
Vertical length of 1st sacral foramen ..	0·19	1·33
Vertical length of 2nd sacral formaen ..	0·17	1·29
Vertical length of 3rd sacral foramen ..	0·17	1·20
Vertical length of 4th sacral foramen	0·14	0·74
Height of symphysis ..	0·73	3·16
Thickness of iliac bar overlying the sacrosciatic notch ..	0·98	..
Number of sacral pieces articulating with ilium ..	3	2
Height of pelvis ..	1·56	12·3

The opening of the ureters into the bladder is seen above the level of the openings of the Wolffian ducts.

The gonads exhibit the epithelial cords identical with the testes.

The levators prostatæ and the puboprostatic ligaments are visible.

The levatores are well formed between the posterior surfaces of the bodies of the pubes and the ischial spines.

The coccygeus muscle is also well differentiated.

100 *mm. Pelvis*

In this embryo a fuller growth of the cartilagenous pattern of the pelvis and the well formed intrapelvic joints are depicted.

In this specimen the upper portions of the iliac cartilages project backwards beyond the cartilaginous discs of the sacral vertebræ (Fig. 7).

The sacral foraminæ are much larger than those found in the 42 mm. specimen.

Centres of ossification are clearly discernible in the bodies of all the sacral vertebræ. The neural arches have commenced to unite.

The sacro-iliac joint.—The sinuous surfaces of the alæ of the sacrum are seen.

The upper two pieces of the sacrum articulate with the posterior medial surface of the ilium on each side.

Thin fibrous strands pass in between the joint surfaces while blood vessels from the vertebral canal as well as vessels lying in front of the sacral vertebræ supply the joint.

The nutrient branch to the ilium supplying the cranial medial segment of the ilium is visible.

The anterior and posterior sacro-iliac ligaments are clearly seen.

The greater sciatic notch is clearly visible.

The supra-pubic ramus is also exhibited with the obturator foramen below it.

The depth of the symphysis is calculated by multiplying the thickness of the section with the number of sections exhibiting the symphysis pubis (Table I A).

The depth of the pelvis is calculated by multiplying the thickness of the sections with the number of slides between the first slide showing the ilio-pubic junction above and the lowest part of the ischium below.

Ossification has started in the ilium above the greater sciatic notch and also over the ischium along its lower and medial part.

The urogenital ducts.—The fimbrial ends of the Mullerian ducts are seen on both sides attached to the posterior wall by the urogenital mesentery. The ovaries are medial to the tubes (Fig. 7).

The Mullerian ducts fuse lower down where the lumen of the fused ducts exhibit the shape of an elongated S running transversely (Fig. 6).

The lumen becomes transversely oval and the mesoderm of the genital cord enveloping it becomes denser. The utero-sacral ligaments can be noticed.

Still lower down, the lumen of the fused utero-vaginal canal disappears.

The Wolffian ducts disappear at the level of the 2nd sacral vertebra.

The external urethral opening is visible.

The rudiments of the paraurethral glands are seen (Fig. 8).

The pubovaginalis and pubo-rectalis are clearly seen as they pass around the vaginal core and anal canal respectively.

The region of the urogenital sinus flanked by the bulbs of the vagina and the bulbospongiosus external to the vaginal bulbs is seen.

Into the walls of the sinus on either side, just posterior to the bulb, open the ducts of the Bartholin's glands (Fig. 9).

The proctodeum is continuous with the rectum.

A cursory examination was made of the slides depicting the 9 mm. and 14 mm. C.R.L. embryos. The 9 mm. embryo exhibited a mesodermic condensation around the acetabular region of the iliac blastema.

The serial sections of the 14 mm. embryo disclosed the posterior growth of the iliac blastema directed towards the vertebral column.

Micrometry of the pelves in the 42 *mm. and* 100 *mm. specimens.*—Under the miscroscope the measurements of the several functions of the pelvis in the 42 and 100 mm. C.R.L. stages were taken with the help of a micrometer. The measurements are appended.

The Wax Model Reconstruction

From these wax models a few observations have been made.

The 42 mm. embryo.—Its sacro-iliac articulation (the upper 3 sacral vertebræ articulate with the ilium); the patterns of the testes and the genito-urinary ducts point to a male embryo as observed in the serial sections under the microscope.

100 mm. specimen.—The nature of its sacro-iliac joint (upper two vertebræ articulate with the ilium), the patterns of the Mullerian ducts, the Wolffian ducts and the gonads point to a female pelvis, as observed in the serial sections under the microscope.

The wax reconstruction models exhibit the following features in each of these specimens (Figs. 10 and 11).

	42 mm.	100 mm.
Curvature of Sacrum ..	Uniform	Curvature is noticed in the lower part
Pubic region ..	The epipubis is observed as a raised eminence on the upper border of the symphysis	The epipubic eminence is observed

The false pelvis is not well formed in proportion to the true pelvis in the 42 mm. specimen.

In the 100 mm. specimen the portions of the pelvis above the pelvic brim are well developed.

In the 42 mm. specimen the greater sciatic notch exhibits the narrow type, *i.e.*, the male type. It is wider in the 100 mm. pelvis and this relates to the female type.

The ischial portion of the pelvis is comparatively not well fashioned out in the 42 mm. and the ischial spine is not formed. In the 100 mm. specimen the ischial spine is well formed and is of the medium type and the ischial portions of the pelvis are clearly fashioned. There is a thickening over its lower surface suggesting the hypoischium.

The inlet.—The promontory in the 100 mm. specimen is less projecting as compared with that in the 42 mm. specimen.

The posterior segment is shorter in the 42 mm. specimen.

In the 100 mm. pelvis the inlet is of the anthropoid type with the promontory receding; the wider alæ with the posterior part of the linea

terminalis smoothly rounding off to join the promontory. It presents the anthropoid type of inlet which is not a typical male type (Fig. 11).

DISCUSSION

In the 4·5 mm. embryo the lower limb bud is orientated immediately lateral to the intermediate cell mass. It is possible that the intermediate cell mass possesses an organising power over the lower limb bud and its associated limb girdle. The association of genital and urinary anomalies is an established fact; malformation of the pelvis is also found to occur alongside these anomalies (Laurence Nharton, 1947).

In the 16·1 mm. embryo the pre-cartilaginous formation of the posterior segment is seen. Over the anterior segment it is still a mesodermal condensation. The posterior medial margins of the ilium have well flanked the first three pieces of the sacrum. At this stage the gonad is just commencing to differentiate and the Mullerian ducts have commenced growing downwards. It can be assumed hypothetically that the number of vertebral segments articulating with the ilia and the early formation of the posterior segments of the ilia are controlled by the chromosomal constitution of the fœtus established at fertilisation. These organisers affect also the general architecture of the body and especially the early constitution of the pelvis thus obtaining a mono-sexual identity of pattern.

The 23 mm. embryo is interesting for its abnormality. The lumbo-sacral region is deviated from the middle line towards the right. The vertebral bodies have undergone torsion. The right iliac bone is on a higher plane than the left and the acetabulum on the left is situated well forward anterior to an imaginary transverse line passing through the centre of the right acetabulum.

The section of the bladder is tilted from a straight transverse axis. Its right corner is on a lower level than the left and the urethra commences from the right corner of the bladder section. The defective development of the allantoic stalk and hind gut have probably interfered with the incurving of the tail end of the embryo. The normal formation of the vertebral column and the correct alignment of the archenteron and amnion must be a prerequisite for the normal growth of the pelvis (Thomas H. Bryce, 1895).

The 42 mm. pelvis is almost well formed in cartilage. The limb bud has completed its medial rotation. The cartilaginous pelvis as featured in the wax reconstruction model has not attained the proportionate height and depth of the adult pelvis. The ischio-pubic ramii are not well formed.

The acetabulæ are shallow. The pubic symphysis shows the correct adult pattern. The urogenital folds have met in the pelvis and within the genital cord. The Wolffian ridge has attached itself to the anterior abdominal wall by the inguinal fold. The fibroblasts within this fold have become continuous with those of the inguinal and pre-pubic regions. The formation of the mesodermal tissues of the pubic portion of the pelvis and the inguinal regions seem to be concomitant results obtained from the hormonal influences of the growing gonad (Keith, 1948). Along with these changes is also occurring the downward growth of the Mullerian system.

The 100 mm. pelvis conforms to the adult pattern. The vaginal epithelial core is formed. The fused Mullerian ducts are acquiring a mesodermal coat. The depth and height of the pelvis are well acquired in the 100 mm. stage. The developmental formation of the lower anterior pelvis and the period of development of the vagina (prostrate in the male) seem to be interlinked. The pelvic floor is begnining to take shape and the ischial spine shows prominence. The side walls of the pelvis relating to its depth are growing and the white line is markedly prominent which seems to occur simultaneously with the formation of the pelvic floor. The pubo-vaginalis and pubo-rectalis are clearly seen in the 100 mm. specimen as they course around the future vaginal wall and the recto-anal junction.

Conclusions

1. The axis of the pelvis is laid with the formation of the vertebral column.

2. The blastema of the acetabular region is formed next by the 9 mm. stage.

3. The sacro-iliac articulation is completed by 16·1 mm. stage, *i.e.*, before the gonads are completely differentiated into specifically male or female type. This stage determines the formation of the posterior segment of the pelvis.

4. A torsion of the vertebral column in early development will result in the malformation of the pelvis.

5. The descent of the gonad and the union of the urogenital folds within the genital cord occur at the 42 mm. C.R.L. stage, after sex differentiation of the gonad is established. At this stage the cartilaginous brim of the pelvis and the full formation of symphysial region occur.

6. At the 100 mm. C.R.L. stage the Mullerian ducts have united; the Fallopian tubes and ovaries have found their definitive positions. The

pelvic floor, with its fasciomuscular layers relating to the upper pelvic floor, the levator ani coccygeus and the sphincteric muscles of the urogenital and anal regions are well differentiated. The vagina at this stage is not canalised.

This investigation was a part of the work executed by me during my stay in Glasgow between March 1948 and May 1950. I am grateful to the Government of India as well as to all my superiors through whose offices I was able to procure the scholarship for undergoing my post-graduate studies in Glasgow.

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Explanation of Plates

Plate XXIV

Fig. 1. Transverse section of the 16·1 mm. C.R.L. human embryo at the level of the acetabulum, showing a process of the ilium directed backwards towards the 1st sacral vertebra.

Fig. 2. Transverse section of the 16·1 mm. C.R.L. human embryo depicting the posteromedial segment of the cartilage of the ilium articulating with the 3rd sacral vertebra.

Plate XXV

Fig. 3. Transverse section of the upper pelvic region of the 23 mm. C.R.L. human embryo showing:

(1) The torsion of the bodies of the vertebræ.

(2) The tilting of the bladder.

(3) The shelf-like projection on the left side.

(4) The rotation of the pelvis.

Fig. 4. Transverse section of the 42 mm. human embryo at the level of the symphysis pubis. The pubic cartilages meet at the symphysis.

Plate XXVI

Fig 5. Transverse section of the 42 mm. C.R.L. human embryo in the mid-pelvic region to show the Wolffian ducts opening into the urogenital sinus on either side of the Mullerian tubercle. The vaginus masculinus is seen in between Wolffian ducts at their entry into the urogenital sinus.

Fig. 6. Transverse section through the 100 mm. human embryo depicting the top portions of the iliac cartilages. The fimbriated ends of the Mullerian ducts are seen laterally in the pelvic cavity. The ovaries lie to the medial side of the fimbriated ends of the Mullerian ducts.

Fig. 7. Transverse section of the 100 mm. C.R.L. embryo over the symphyseal region.

The Mulierian ducts have fused in the middle to form an S-shaped lumen.

The pattern of the symphysis pubis is seen in front of the bladder. The cartilaginous ischial region is seen on either side of the cartilaginous bodies of the pubes.

Plate XXVII

Fig. 8. Transverse section of the lower pelvic region of the 100 mm. C.R.L. human embryo; only the paraurethral ducts are depicted.

Fig. 9. Transverse section of the 100 mm. C.R.L. human embryo depicting only the area of Bartholin's glands.

Plate XXVIII

Fig. 10. A photograph of the wax reconstruction model of the 42 mm. pelvis. The height and depth of the pelvis are not proportionate to the width of the pelvis at this stage.

Fig. 11. A photograph of the wax reconstruction model of the 100 mm. pelvis. A relative increase in the depth of the pelvis may be noted. The full height of the pelvis could not be obtained due to absence of serial sections in the cephalic region of the pelvis.

Abbreviations

B.S., Body of Sacrum.
P.Pr.I.C., Posterior Process of Iliac Cartilage.
R.Ut.F., Recto Uterine Fossa.
F.M.D., Fused Mullerian Ducts.
S.Pub., Symphysis Pubis.

P.M.Seg., Posterior Medial Segment.

3rd P.S., 3rd Piece of Sacrum.
Il.R., Ilium Right.
Il.L., Ilium Left.
B.V., Bodies of Vertebræ.
Il., Ilium.
G., Gonad.
Bl., Bladder.
Urg.S., Urogenital Sinus.
H.g., Hind Gut.

F. End M.D., Fimbriated End Mullerian ducts.
Ov., Ovary.
Urg.S., Urogenital Sinus.
Par.Ur.D., Paraurethral Duct.
S.P.A., Sub Pubic Angle.
S., Sacrum.
Int.V.D., Intervertebral Disc.
Isch., Ischium.
Obt.f., Obturator foramen.
W.D. Wolffian duct.
Bar.D. α-Bartholin's duct.

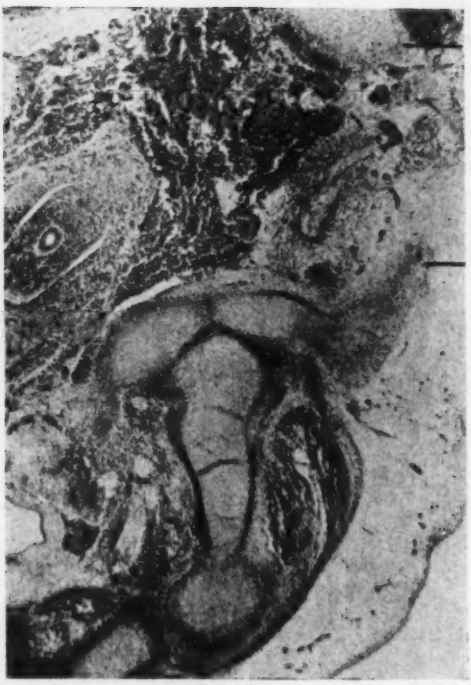

FIG. 1

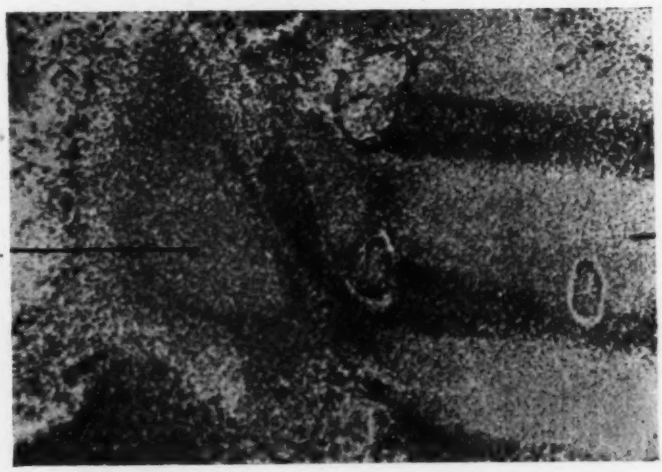

FIG. 2

FIG. 3

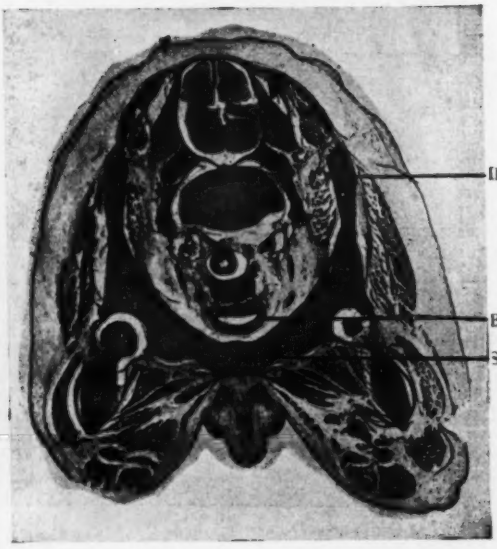

FIG. 4

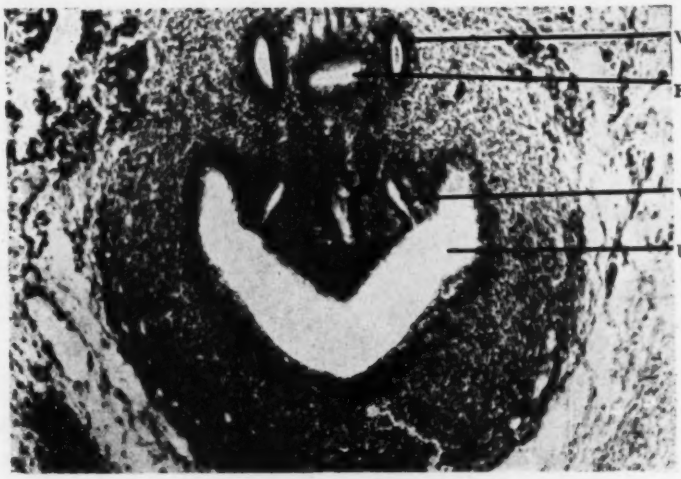

FIG. 5

FIG. 6

FIG. 7

FIG. 8

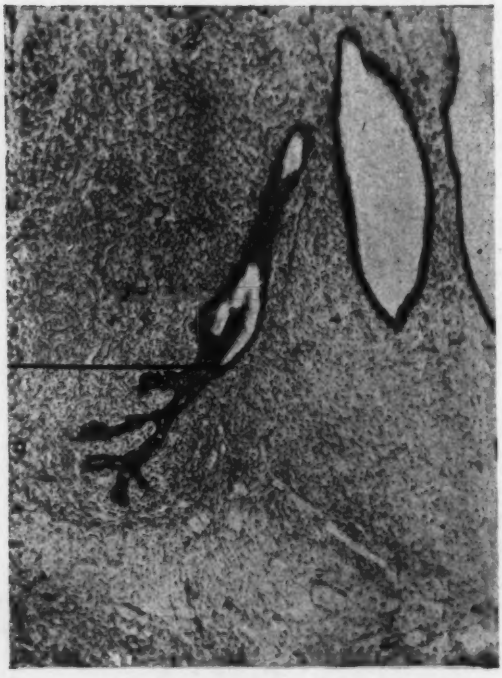

FIG. 9

FIG. 10

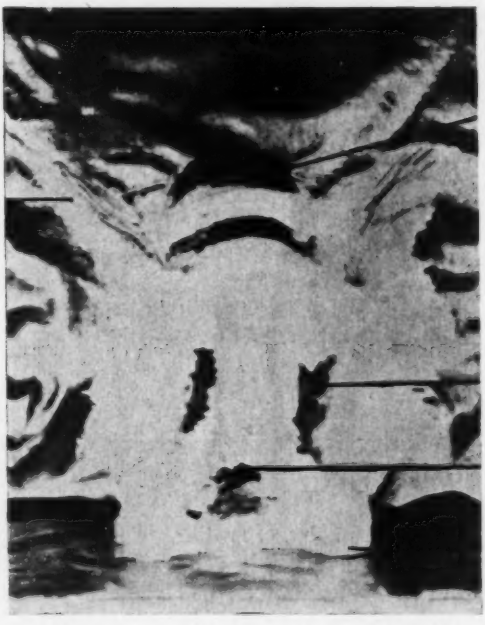

FIG. 11

CONTRIBUTIONS TO THE CYTOLOGY OF INDIAN ANURA (AMPHIBIA) : A STUDY OF CHROMOSOMES IN THE TWO SEXES OF *BUFO MELANOSTICTUS* SCHNEIDER (BUFONIDÆ)

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Introduction

The family Bufonidæ of the suborder Procœla has been cytologically well explored and the chromosome numbers of about 14 species of *Bufo* are so far known to us (Makino, 1951; Wickbom, 1949 *a*, *b*). Of these only one Indian species, *viz.*, *Bufo stomaticus* has been studied (Asana and Mahabale, 1941). In view of our scanty knowledge of the cytology of Indian Bufonidæ, the present investigation was undertaken and a comparative study has been made of both the male and female karyotypes of the common South Indian species *Bufo malanostictus* Schneider.

The chromosomes have been studied mainly from the spermatogonial and oogonial metaphase plates. The study of the oogonial chromosomes was made from acetocarmine squashes of the ovary. The preparation of good squashes of the ovary presents technical difficulties partly on account of the large quantity of yolk and partly due to the rarity of the suitable stage. Hence, a large number of squashes had to be prepared in order to obtain clear preparations of the required stages.

OBSERVATIONS

1. *Spermatogonial metaphase.*—Figures 1 and 3 represent a spermatogonial metaphase plate in polar view with 22 metacentric chromosomes. All of them are V-shaped with equal arms and the homologues lie close to one another. The 11 pairs of chromosomes which constitute the complement are shown serially arranged in Fig. 5 from which it may be noted, that of the 11 pairs, 6 are large and 5 are small. All of them have median centromeres.

2. *Oogonial metaphase.*—Figures 2 and 4 show the polar view of an oogonial metaphase plate and the number of chromosomes can be clearly made out to be 22. The larger chromosomes are seen towards the periphery of the plate surrounding the smaller ones. Fig. 6 represents a serial arrangement of the 22 chromosomes that resolve into 11 homologous pairs. Here also the division of the chromosomes into 2 sets (one large and one small) could very well be seen. All the chromosomes have median centromeres only.

In brief, a study of the spermatogonial as well as the oogonial metaphase chromosomes of *Bufo melanostictus* shows the following features, common in both: (i) the number of chromosomes, *i.e.*, 22, (ii) all the chromosomes are metacentrics only, and (iii) the chromosomes fall into two well-defined groups, *i.e.*, 6 pairs are large and the rest are small.

DISCUSSION

Asana and Mahabale (1941) have also reported that $2n = 22$ in *Bufo stomaticus*, the only other Indian species so far studied but they say that 7 pairs of chromosomes are large and 4 are small. In *B. melanostictus*, however, there are definitely 6 pairs of large and 5 pairs of smaller chromosomes in both the male and the female karyotypes. Unfortunately, a more detailed and critical comparison with the findings of the previous investigators could not be made, as a detailed account of the individual chromosomes of *Bufo stomaticus* has not been given by them.

The diploid chromosome number of 22 with 6 large and 5 small pairs of chromosomes has been found to be of uniform occurrence in all the European, North American, South American, African and other Asian species of *Bufo* (Wickbom, 1949 *a*, *b*; Makino, 1951). Even the two exceptions to this condition reported by King (1907) and Poska-Teiss (1933) were later proved to be erroneous by Witschi (1933) and Saez *et al.* (1936), respectively. The striking cytological uniformity and stability of the genus *Bufo*

despite its wide geographical distribution has been pointed out by Wickbom (1949 *a*). This view is further confirmed by the present investigation.

A comparison of the chromosomes of the male and female karyotypes of *B. melanostictus* shows very clearly that there do not occur any heteromorphic pair or pairs of chromosomes in them. All of them are only regular chromosome pairs without even slight inequality in any pair of any karyotype. It is thus definite that the sex chromosomes which can be identified at the morphological level are absent in the form now studied. After a more thorough and critical study, Wickbom (1945) concluded that "in Anura and Urodela nothing has been brought to light which even suggests the occurrence of morphologically or behaviourly recognisable heterochromosomes in either sex". Wickbom's opinion has been endorsed by Matthey (1949, 1951) also, who says that among Amphibia "Sex heterozygosis does not find any morphological expression". The present investigation goes to strengthen this view further.

Summary

1. The chromosomes of both the male and female forms of *Bufo melanostictus* have been studied mainly from the acetocarmine squashes of the testis and the ovary.

2. In both the karyotypes, the chromosome number is 22, all the chromosomes are metacentrics and 6 pairs of chromosomes are large and 5 are small. In other words, the number of chromosomes, their morphology and their division into two well-defined sets are common features shared by the karyotypes of both the male and the female forms.

3. The above findings support the view that sex chromosomes are cytologically not recognisable in *Bufo melanostictus*.

Acknowledgements

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EXPLANATION OF PLATE

FIG. 1. Photomicrograph of spermatogonial metaphase plate—polar view—Acetocarmine squash, × *ca.* 2,500.

FIG. 2. Photomicrograph of oogonial metaphase plate—polar view—Acetocarmine squash, × *ca.* 2,500.

FIG. 3. Spermatogonial metaphase plate—drawing, × *ca.* 4,000.

FIG. 4. Oogonial metaphase plate—drawing, × *ca.* 3,500.

FIG. 5. Homologous pairs of spermatogonial chromosomes serially arranged in order of size, × *ca.* 5,000.

FIG. 6. Homologous pairs of oogonial chromosomes serially arranged in order of size, × *ca.* 3,500.

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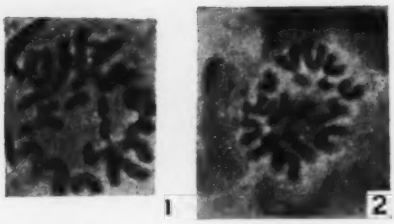

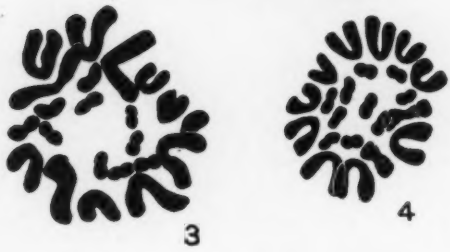

FIGS. 1–6

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